EVALUATION OF THE NATURAL BIODEGRADATION OF AIRCRAFT DEICING FLUID COMPONENTS IN SOILS

Laura M. Johnson, Captain, USAF
AFIT/GEE/ENV/97D-12

DEPARTMENT OF THE AIR FORCE
AIR UNIVERSITY
AIR FORCE INSTITUTE OF TECHNOLOGY

Wright-Patterson Air Force Base, Ohio

Disclaimer Statement

The views expressed in this thesis are those of the author and do not reflect the official policy or position of the Department of Defense or the United States Government.

AFIT/GEE/ENV/97D-12

EVALUATION OF THE NATURAL BIODEGRADATION OF AIRCRAFT DEICING FLUID COMPONENTS IN SOILS

THESIS

Laura M. Johnson, B.S.

Captain, USAF

December 1997

Presented to the Faculty of the School of Engineering

of the Air Force Institute of Technology

Air University

In Partial Fulfillment of the

Requirements for the Degree of

Master of Science in Engineering and Environmental Management

Prof. Daniel E. Reynolds

Dr. Mark N. Goltz, PhD

Dr. Charles A. Bleckmann, PhD

Chairman

AFIT/GEE/ENV/97D-12

EVALUATION OF THE NATURAL BIODEGRADATION OF AIRCRAFT DEICING FLUID COMPONENTS IN SOILS

THESIS

Presented to the Faculty of the School of Engineering
of the Air Force Institute of Technology
Air University
In Partial Fulfillment of the
Requirements for the Degree of
Master of Science in Engineering and Environmental Management

Laura M. Johnson, B.S.
Captain, USAF

December 1997

Acknowledgments

First and foremost, I with to thank my thesis advisor, Dr Charles Bleckmann for his advice, guidance, and motivational support. He helped me to understand not only the microbial biodegradation process but also the research process and the scientific principles behind this work. Together we solved many "simple" problems.

I also express sincere thanks to Professor Dan Reynolds, who provided me with the analytical tools necessary to evaluate and organize my data statistically. Without his help and guidance, I would have been completely lost.

Thanks also go out to Dr Mark Goltz, who provided many important inputs to my thesis. Thanks for helping to get my thoughts straight and in organizing my thesis in a logical manner.

I would also like to thank Major Ed Heyse, who provided me with an increased understanding of the principles behind high performance liquid chromatography (HPLC) and was always available and willing to help when I had a question. Maj. Heyse, thanks for your advice and help along the way.

Thanks also need to go out to Dan Mika and David Shepherd, who have helped me tremendously in the lab. Their help with the HPLC saved me an unimaginable amount of time. Dan and David, thank you very much.

Thanks to Major Jeff Cornell, University of Colorado - Boulder. This research was also done to support his research on a land treatment system for aircraft deicing fluids. Thank you for suggesting the topic and for providing much needed background information on tolyltriazole.

Thanks to PMC Specialty Group, Cincinnati, OH, from whom I received the tolyltriazole and background information. Thanks specifically to Zetta Bouligarki who was more than willing to help me out and get any information and materials that I needed.

Lastly, I would like to thank Lt AnnMarie Halterman-O'Malley. Although we did separate theses, I considered you my partner in this process and could not have done it without you. Together we overcame many obstacles, that would have been difficult to do alone. Your support, patience, and most of all your friendship, has meant a lot to me. Thank you.

Laura Johnson

Table Of Contents

List Of Figures

List Of Tables

AFIT/GEE/ENV/97D-12

Abstract

This research effort was conducted to analyze the biodegradation of propylene glycol (PG) and tolyltriazole in two different soil types; a sandy soil and a high clay soil. Both an automated respirometer and a high performance liquid chromatograph (HPLC) were used in the analysis. Two separate experiments were conducted. In the first experiment, one level of tolyltriazole was added to the soils to determine whether or not there was a difference in the biodegradation rates of tolyltriazole in the two soils. The respirometer results indicated that there was a significant difference between the respiration rates of the microorganisms in the two soil types, and the HPLC results indicated that biodegradation of the tolyltriazole was occurring in the microcosms. In the second experiment, only the high clay soil was used since it had a significantly higher respiration rate than the sandy soil. This experiment was conducted to determine the affect (inhibition, stimulation, or no effect) of a combined treatment of tolyltriazole and PG vs. the contaminants acting by themselves. The soil was treated with tolyltriazole alone, PG alone, and a combined mixture of the two. One level of PG was used throughout, and two levels of tolyltriazole were used, for a total of five different treatments. Both the respirometer and HPLC results indicated that biodegradation was occurring. The respirometer results indicated that there was a significant increase in the respiration rates of the microorganisms when the contaminants were mixed vs. by themselves, thereby indicating an increase in biodegradation. The HPLC results, however, indicated that the same amount of tolyltriazole was biodegrading whether it was in combination with PG or acting alone. These results may indicate that the significant increase in respiration was due to an increase in biodegradation of PG.

EVALUATION OF THE NATURAL BIODEGRADATION OF AIRCRAFT DEICING FLUID COMPONENTS IN SOILS

I. Introduction

1.1 Overview

Aircraft deicing/anti-icing fluids (ADAFs) are used worldwide in considerable quantities to remove and prevent accumulation of snow, ice, and frost from aircraft. It has been estimated that approximately 3,785 L (1,000 gal) of ADAF is used to de-ice a typical large passenger jet (21:40). Although the main component of ADAFs are glycols, which are readily mineralized to carbon dioxide and water, they are still a problem to the environment because of their high oxygen demand (27:23). Since most ADAF formulations are proprietary, their exact composition isn't always available, so determining their environmental impact is difficult. Many ADAFs contain a chemical used as a corrosion inhibitor, tolyltriazole; however, little is known about its environmental fate and/or how it biodegrades. This study measures the effects of soil type on the biodegradation of tolyltriazole and the effects of tolyltriazole on the biodegradation of propylene glycol (PG), the main component of ADAFs.

1.2 Problem

Applying ADAFs to aircraft is common practice in cold weather regions, and along with its use comes environmental concerns. Because ADAFs are used in the winter when the ground is frozen, much of the ADAF contacts soil as runoff, either immediately or during a snowmelt. It is estimated that 80% of the fluids are deposited on the ground due to spray drift, jet blast, and wind shear during taxiing and takeoff (11:137). Much of this runoff makes its way into storm water sewers and is ultimately deposited in local surface waters, where it exerts an extremely high biochemical oxygen demand (BOD). The high BOD is of primary concern since it results in the rapid depletion of the dissolved oxygen in the surface water, suffocating the aquatic life (14:875). The carbonaceous BOD (CBOD5) of propylene glycol (PG) is around 1×10^6 mg/L, whereas untreated domestic wastewater is in the range of 200-300 mg/L (21:40). Other concerns include the toxicity of ADAF components to aquatic and mammalian organisms.

Many airports now collect and send the ADAF waste to wastewater treatment plants. Although this is an effective method of treatment, it is very expensive. Because the high BOD associated with the biodegradation of ADAF can wreak havoc on a wastewater treatment plant, the fluid has to be diluted to <10% before municipal facilities will accept it for treatment. Many facilities specify between 1 to 5% glycol as the maximum concentration that they will accept (33:266). Because the volume of ADAF used to de-ice a typical large passenger jet (approximately 3785 L) has a $CBOD_5$ equivalent to the daily domestic

wastewater generated by 5000 people, the waste has to be significantly diluted (21:40). To the airport, this means large volumes of waste being sent to a facility, and large costs, especially if the waste is not within the specified concentration limits. More recently, practices including recycling and on-site degradation of the waste are proving to be more cost effective than sending it to a municipal treatment facility (33:266).

1.3 Research Objective

The purpose of this research was to evaluate the biodegradation of ADAF components under natural conditions using standard respirometry techniques and high performance liquid chromatography (HPLC). Tolyltriazole was analyzed in two different soil types, while mixtures of PG and tolyltriazole were analyzed in one soil type. Oxygen consumption and carbon dioxide production, measured by the respirometer, were used to determine microbial metabolism. The HPLC was used to determine the amount of tolyltriazole left in the soil once the respirometer experiment was complete. The results of this analysis will be used to further the research being conducted by Ph.D. student Major Jeff Cornell and Dr Mark Hernandez at the University of Colorado-Boulder. Their research is aimed at finding ways to manage ADAFs by designing ADAF treatment systems.

1.4 Scope

This study followed many of the same procedures as those of Baker (1995) and Totten (1995) in their studies of the biodegradation of jet fuel JP-8 in various soils using respirometry. This study simulated initial spill conditions by introducing fresh PG and/or tolyltriazole into uncontaminated soils. Two different soil types were chosen, based on their different physical structure (particle size distribution), and organic content. Other than the organic content, the chemical makeup of the soils was very similar. Both soils were taken from areas believed to be free of pollution. The soils were kept to as close to a natural state as possible by minimizing the processing. Respiration was measured in microcosms containing both contaminated and uncontaminated soils. The uncontaminated soil was used as a control to determine the amount of background respiration of the soil. Aerobic conditions were initially established in the sealed microcosms and then automatically maintained by the respirometer.

Two experimental runs were made, each with a different configuration. Experiment 1 analyzed the biodegradation of tolyltriazole in two different soil types while experiment 2 analyzed the effects of two concentrations of tolyltriazole on the same concentration of PG in one soil type. More detail on the experimental setups can be found in Chapter 3. Both experiments were run for approximately 2 weeks, which allowed for the biological activity to peak and then generally stabilize. Samples of soil were taken from some of the microcosms at the end of each experimental run for chemical analysis. Extractions from the soil

were analyzed with the HPLC to quantify the amount of tolyltriazole present. Attempts to analyze PG with the HPLC proved unsuccessful and therefore, analyses of PG extracted from the soil were not a part of this study. No attempt was made to identify the type of microorganisms (bacteria, fungi, etc.) in the soil.

1.5 Terms Used in this Study

Aerobic - Having molecular oxygen present; growing in the presence of air (7:18)

Anaerobic - Living, active, or occurring in the absence of free oxygen (7:40)

Aromatic compound - Benzene and compounds that resemble benzene in chemical behavior. Their ring structure and stable bonds allow them to be resistant to degradation. These molecules contain delocalized clouds of resonant π-electrons and they favor substitution rather than addition reactions, both of which contribute to their stability (24: 322)

Biochemical Oxygen Demand (BOD) - The amount of molecular oxygen utilized by microorganisms in wastewaters, effluents, and polluted waters for the biochemical degradation of organic material and the oxidation of inorganic material. BOD determination is an empirical test that utilizes standardized laboratory procedures and is conducted over a specified time period (usually 5 days) (5:27).

Biodegradation - The breakdown of organic compounds by microorganisms.

Field Capacity - The maximum amount of water that an unsaturated zone of soil can hold against the pull of gravity (6:639).

Heterocyclic - A organic compound, characterized by, a ring composed of atoms of more than one kind (7:533)

Metabolite - a substance essential to the metabolism or a particular organism or to a particular metabolic process (7:715)

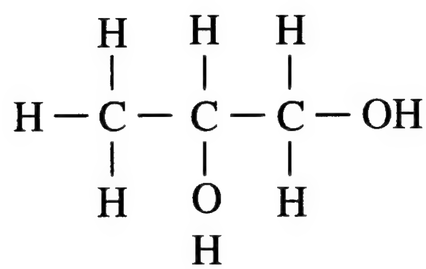
Micro-Oxymax respirometer- An indirect closed loop respirometer designed to detect extremely low levels of oxygen consumption and carbon dioxide production for a variety of studies involving bacteria, insects, plants, cell structures, food, and chemical oxidation (23:3).

Mineralization - The complete transformation of organic compounds into inorganic products (CO_2 and H_2O) (19:110).

Natural Attenuation - The oxidation or breakdown of a substance through natural processes.

Propylene Glycol (PG) - Chemical used in aircraft deicing/anti-icing fluids; $C_3H_8O_2$. See Figure 1-1 below for structure.

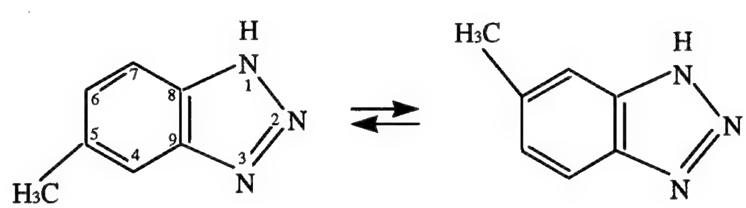
FIGURE 1-1 - Propylene Glycol



Statistical hypothesis - a claim about the value of a single population characteristic, or about the values of several characteristics (4:304).

Tolyltriazole - Chemical used as a corrosion inhibitor in aircraft deicing/anti-icing fluids; $C_7H_7N_3$. See Figure 1-2 below for structure.

FIGURE 1-2 - Tolyltriazole



5-Methyl-1H-Benzotriazole 6-Methyl-1H-Benzotriazole

II. Literature Review

2.1 Background

There are two classes of commercial ADAFs, Type I and Type II. Type I is a relatively thin liquid comprised primarily of glycols and water and is typically used to de-ice an aircraft that already has snow and/or ice buildup. Type II is a more viscous fluid comprised of glycols, additives, and water and is typically used as an anti-icer. The viscous nature of Type II causes it to cling to the aircraft longer than Type I; thereby protecting the surface of the aircraft longer. Many times, Type I and Type II are used in conjunction with one another. Both types eventually drop off of the aircraft and onto the runway when shear stresses are produced during takeoff (21:38).

Most ADAFs are proprietary, thus their exact chemical formulations are unavailable. This proprietary nature means that the composition of ADAFs vary, depending on the manufacturer. This lack of information can make it difficult to relate environmental effects to the presence of specific chemical agents (2:1; 29:314). Although the exact composition may be unavailable, the three main components of an ADAF include glycols, additives, and water. Most ADAFs contain between 50-90% ethylene, propylene, or other types of glycols. Additives such as wetting agents, corrosion inhibitors, surfactants, thickeners, and other agents used to meet performance criteria make up between 10-20% of the ADAF. The remaining portion of the ADAF is water (2:1).

PG is a common industrial chemical. Along with its use in ADAFs, it is used as a preservative and emulsifier in food and bath products. PG-based ADAFs are currently the most common ADAFs in use and the only type authorized for use in the Air Force. More than 745 million pounds were produced in 1991 (33:1). PG is effective in ADAFs because it lowers the freezing point of water to -59°C (27:22). PG is not a known carcinogen or teratogen, and is not considered very toxic to mammalian or aquatic organisms (Oral rat LD_{50}=20,000 g/kg, *Ceriodaphnia dubia* 48hr LC_{50}=18.340 mg/L, and *Pimephales promelas* 48hr LC_{50}=>62,000 mg/L (29:314; 20:3).

Tolyltriazole, a common additive, is used in many products as a corrosion inhibitor. Besides its use in ADAFs, it is used in circulating cooling systems, wrapping tissue and box boards, cleaners, corrosion prevention coatings, and functional fluids such as hydraulic fluids, metal working fluids, specialty lubricants, and automotive coolants (29). Although it can be found in liquids at concentrations between 0.1 to 2.0%, its concentration in ADAFs is usually around 0.2 to 0.5% (29; 3). Tolyltriazole passivates corrosion by forming a barrier film on the surface of metals (29). Although tolyltriazole is not considered a carcinogen and is not very toxic to mammalian organisms unless taken orally (LD_{50} rat = 675 mg/Kg), it is fairly toxic to aquatic organisms (Bluegill Sunfish 96hr Tlm=31 mg/L, Minnow 96hr Tlm=25.5 mg/L, Trout 96hr LC_{50}= 21.4 mg/L, and *Daphnia magna* 48hr LC_{50}=73.7 mg/L) (30).

The last couple of decades have seen many changes regarding the use of ADAFs. The regulations governing the discharge of ADAFs fall under the Clean Water Act, which has its origins in the Federal Water Pollution Control Act of 1972. The Act of 1972 required the Environmental Protection Agency (EPA) to set nationwide effluent standards on an industry-by-industry basis, and established the National Pollutant Discharge Elimination System (NPDES) permit program (8:135). Under the NPDES program, a permit issued by the EPA or authorized state is required if a pollutant is to be discharged from a point source to waters of the United States (8:140). Prior to 1987, storm water discharges were not considered point sources; however, the Water Quality Act of 1987, required the EPA to regulate storm water discharges "associated with industrial activities" by October 1, 1994. Under the EPA's storm water program, all discharges associated with industrial activities, which includes airports, require a NPDES permit (8:155).

As a result of these regulations, airports are now taking a more active role in monitoring and controlling the fate of the ADAFs they use. New airports are being designed and constructed with collection and recycling systems from the beginning, while older airports are altering their operations to meet the requirements. Although many airports send their waste to local wastewater

treatment plants, many are finding that it can be more cost effective to recycle and provide on-site degradation of the waste.

Another major change that has occurred within the last 5 years has been the shift from ethylene glycol (EG) based ADAFs to propylene glycol (PG) based ADAFs. A national shortage of EG occurred during the winter of 1994 due to the high amounts of snow and ice that winter. The supply of EG based ADAFs couldn't keep up with the demand, so a PG based alternative was substituted. The substitute was so effective that it captured the market (13:43). Solutions of EG and PG push the freezing point of water down to -13°C and -59°C respectively (27:22). Because PG based ADAFs are less toxic to aquatic and mammalian organisms than EG, they are considered more environmentally friendly, and are now the preferred ADAF. EG is also listed under CERCLA as a hazardous substance and is therefore subject to the Emergency Planning and Community Right to Know Act (EPCRA) (12:1). As part of a major AF initiative to use only environmentally friendly fluids, the Air Force (AF) had made the switch to PG prior to the winter of 1994. On March 31, 1992, Brigadier General James E. McCarthy, the AF Civil Engineer, directed an immediate USAF-wide prohibition on the use of EG (25).

PG based ADAFs have proven to be just as effective as the EG based fluids in removing snow and ice and are less toxic to aquatic and mammalian organisms.

However, although both are biodegradable, PG degrades slower and has a higher BOD than EG. Thus, it can still be unfriendly to aquatic systems (12).

2.2 Biodegradation

Biodegradation rates are known to be influenced by a variety of physical, chemical, and biological factors. Some of these factors include: the type and size of the indigenous microbial population, the medium in which the contaminant is located, the pH and temperature of the medium, the availability of water, a carbon source, inorganic nutrients such as nitrogen and phosphorous, and an oxygen source or other electron acceptors. Environmental factors will control the size and type of microbial populations present, which in turn will control the rate of biodegradation. Other factors that influence the biodegradation rate, but are not as well understood, include the interactions between various populations of microorganisms, availability of the contaminant to the microorganisms, interaction between the microorganisms and the individual components of the contaminant, the various metabolic pathways, and the metabolic by-products that form and are consumed during the biodegradation.

Biodegradation is considered useful since it oftentimes results in conversion of a contaminant by microorganisms into more environmentally friendly compounds, such as carbon dioxide and water. The usual media in which this process occurs include water, soil, and/or air, while the energy or carbon source used is usually

the contaminant. Different contaminants will be degraded differently. The size and structure of the molecule can play a big part in how readily it degrades. For example, straight chain structures (glycols) are more easily degraded than ring type structures (triazols).

As stated above, the medium in which biodegradation occurs plays an important role. In soil environments, the soil chemistry and structure can affect both the rate and cumulative amount of degradation. Different soil types vary in sorption and ion-exchange properties, organic matter level, micro- and macro-nutrients, as well as microbial populations (9:1278). Water, gasses, organic material, and microorganisms can all be captured between, on the surface, or within the particles which make up the soil matrix. Biodegradation can occur in any of these locations, provided that the size of the spaces are large enough for the microorganisms to penetrate. The soil makeup also affects how the contaminant moves through the soil. Sorption is more likely to occur in a soil with a high organic content vs. a sandy soil with a low organic content. Advection and dispersion are more likely to occur in a more porous sandy soil with a low organic content than a clayey soil with a high organic content. Whether or not the degradation will be aerobic or anaerobic is also influenced by the soil make up and location of the degradation within the soil. Low permeability soils will tend to have more anaerobic degradation than high permeability soils. Anaerobic degradation is also more likely to occur in deeper soil layers where the oxygen availability is lower (19:373).

Soils with a high clay content can have both positive and negative effects on biodegradation. Clay can tend to be fairly impermeable, thereby reducing the oxygen and water available to the microorganisms. It can also immobilize cells, inactivate enzymes, and polymerize certain substrates. The positive effects include enhancing the exchange of enzymes with substrates (caused by the proximity of the cell and the substrate), buffering against wide pH swings, retaining needed moisture, and protecting against predators and toxic metabolites. Clay particles are also important because biofilms, which are thought to be the principal site of microbial activity, tend to form on their surfaces (22:19).

Due to the wide range of conditions in soil environments, diverse microbial populations usually exist; however, bacteria, actinomycetes, and fungi are the principle microorganisms responsible for the degradation of most organic chemicals. Although bacteria are not generally the major component of soil biomass because of their small size, they are the most numerous in soils and have a high metabolic rate. This high metabolism accounts for a significant percentage of the total metabolism in the soil. Bacteria are largely responsible for the elemental transformation of carbon, nitrogen, phosphorus, sulfur, and iron. Fungi are larger in size than bacteria and therefore account for a large portion of the microbial biomass. Because fungi are tolerant to low pHs, they account for a large percentage of the biodegradation in acidic soils.

Actinomycetes, filamentous bacteria, are tolerant to high pHs, so they can be found in basic soil environments (17:130).

Although we know that microorganisms will be present in nearly every environment, biodegradation can be optimized when environmental factors are within certain ranges. Temperature, moisture content, and soil pH are among those factors. Because soil environments can experience wide daily and seasonal changes in temperature, the temperature can have a large impact on the degradation rate. Increases in temperature can influence the volatilization, desorption, and leaching of materials as well as the chemical and biological degradation processes. Moisture content is another important factor affecting the fate of a chemical in the environment. Besides being essential for the life of the microbes, the amount of water affects the availability of contaminant by controlling its movement and sorption. Optimal biodegradation occurs when the moisture content is between 25%-85% of the field capacity (32:7). The pH of a soil can change with depth and with time. The upper horizons in wet climates are usually more acidic than the lower horizons or drier climates because of the combined effects of litter decomposition and the leaching of bases (22:9). This change in pH can eventually change the rate at which biodegradation occurs. Because the biodegradation of different contaminants requires different microorganisms, there are no exact limits for temperature, moisture content, and pH ranges; however, temperatures between 15-45°C, moisture content between

25-85% field capacity, and a pH range of 5.5 to 8.5 are generally accepted as optimal (32:7).

The concentration of contaminant present and the frequency of its occurrence (one time spill vs. reapplication as in the case of ADAFs at airports) controls the kinetics or rate of the biodegradation. Zero order kinetics describe the condition where the growth rate of the microorganisms is independent of the concentration of the contaminant. This situation usually occurs at the beginning of the biodegradation process when the concentration of the contaminant is large relative to the microbial population. First order kinetics describe the condition where the rate of degradation is proportional to the concentration of the contaminant, and second order kinetics apply when the rate of degradation is a function of both the contaminant concentration and the size of the microbial population (17:120). The concentration and type of chemical, along with the microbial population, influence which kinetic expression describes the biodegradation. The microbial degradation of many water-soluble chemicals in soils, however, has been shown to typically follow first-order kinetics (17:133).

The biodegradation process can be as simple as one microbial population mineralizing the contaminant to carbon dioxide and water in one step or, it can be a much more complicated process in which many populations are needed for complete mineralization. The process of biodegradation usually begins after a lag period in which the microorganisms are adjusting to the new contaminant by

producing the needed enzymes. Populations which cannot produce the necessary enzymes will die off and new populations that can will emerge. Microbial populations will rise and fall in conjunction with the conversion of the contaminant into different compounds on its way to mineralization. During the process, the new population will use the previous population's metabolites to further convert the compounds; however, complete mineralization does not always occur. Sometimes, the metabolites of one population can have a toxic effect on another population, thereby significantly slowing down or stopping the process.

Every natural organic compound on earth is susceptible to biodegradation; however, the rate at which it occurs depends on many different factors. Some compounds are very easily degraded and can be mineralized in a few hours or days while others may take much longer, even thousands of years. Although the mineralization of a contaminant may occur in a series of steps, any and all of the activities which influence the biodegradation process can occur simultaneously and within a few microns of each other.

2.2.1 Biodegradation of Glycols

Many studies have been conducted to evaluate the biodegradation of glycols. Glycols are straight chain alcohols with two attached hydroxyl groups (7:487). Although there are many factors which influence biodegradation rates, one of the main considerations in glycol degradation is the chain length and molecular

weight. Because glycol chain length can vary, so can the degradation rates. When studying the biodegradation of Polyethylene Glycol (PEG), Patterson et al. found that the rate and extent of biodegradation decreased with increasing chain length and molecular weight (10:621,623) Glycols can be as simple as ethylene glycol ($C_2H_6O_2$), or can be as complicated as polyethylene glycols, which have the common structural formula of $HO(CH_2CH_2O)_nCH_2CH_2OH$, but differ from each other in their average molecular weight. Polyethylene glycols can have molecular weights up to 20,000 g/mole (16:679).

When propylene glycol biodegrades, intermediate products such as aldehydes and organic acids (lactic, pyruvic or acetic acids) can be formed (20) . These intermediate compounds are produced in small quantities and are quickly degraded to the end products of carbon dioxide and water. Many studies have concluded that most glycols are readily degradable in both the soil and water environments (9, 10, 14, 15, 18, 19, 26, and 33).

2.2.2 Biodegradation of Benzotriazols

One of the benzotriazole derivatives that is commonly used as a corrosion inhibitor, and is of particular interest in this thesis, is 5(6)-methyl-1H-benzotriazole or more commonly known as tolyltriazole (Figure 1-2). The pathway in which benzotriazoles and their derivatives degrade is different than that of the glycol solvents in which they are commonly dissolved. One of the differences in degradation is caused by the fact that they are heterocyclic

compounds rather than straight chain alcohols. Although there is no published data on microbial degradation rates or on the fate of triazoles in the natural environment, it can be expected that triazoles will degrade at a slower rate than glycols due to their more complex structure. The degradation by-products are likely to be an intact triazole ring with two alkyl attachments resulting from benzene ring cleavage (31).

III. Methodology

3.1 Overview of Experiment

This chapter describes how this study was conducted in order to show the rate of biodegradation of aircraft deicing agents in two different soil types. A respirometer and a high performance liquid chromatograph (HPLC) were used to analyze the biodegradation. The respirometer measured the amount of oxygen consumption and carbon dioxide production, which are measures of the metabolism of the microorganisms in the soil. The HPLC was used to analyze soil extracts once the respirometry experiment was complete to determine the amount of contaminant still left in the soil. Both a combination of propylene glycol and tolyltriazole in water and tolyltriazole alone in water were added to the soil to simulate exposure of the soil microorganisms in a land treatment system. The microcosms were kept at 30°C and the headspace gases were monitored every 6 hours for a 2 week period. Through the data collected, increases or decreases in oxygen consumption/carbon dioxide production, which indicate biological activity, could be evaluated.

3.2 Soil Preparation

3.2.1 Purpose

Both a sandy and a high clay soil were chosen so that the biodegradation of aircraft deicing agents could be analyzed in differing soil environments. The soils are important since they contain the nutrients, microflora, gasses, water,

and structure necessary to carry out the biodegradation process. The sandy soil differed from the high clay soil in that both its moisture content and its organic carbon content were less; both of which contributed significantly to the biodegradation process. However, because the high clay soil had a higher organic carbon content, there were more places for the contaminant to sorb to the soil making it less available to the microorganisms. These two differing environments can produce different biodegradation rates. In order to minimize any confounding effects, both soils were processed and handled identically. Although the goal of this work was not to replicate in situ conditions, preparation and handling of the soils was kept to a minimum to keep the soils as close as possible to their natural state.

3.2.2 Soil Collection

The soils were collected from locations that were characteristic of that type of soil. The sandy soil was collected from a recently exposed river bed during a time of low water. Collection was made on a sunny, dry day in May with an ambient temperature of about 18°C. The river runs parallel to and just north of Hwy. 35 in Beavercreek, OH. The point of sampling was about a mile east of North Fairfield Rd. Prior to collection, the area had experienced several weeks of rainy weather, producing mild flood-like conditions. Once the water level receded and the river bed was exposed, a wet sandy soil with some plant root structures was collected.

The high clay soil was collected from a wooded area adjacent to Bldg 470 on Area B, Wright-Patterson AFB, OH. The soil was collected on a sunny, dry day in late April with an ambient temp of about 15°C. The soil collected was moist, dark, and contained some plant root structures.

The collection, handling, and processing procedures for both soils were identical. Surface debris was first cleaned off of the collection area, then the top 10 cm of a one meter square area of soil was removed and discarded. A clean steel shovel was used to remove soil samples down to a depth of about 50 cm. The soil was placed in a clean, 1 gallon plastic bucket for transport back to the laboratory. The soil was then sieved to remove any stones, twigs, roots, and/or other foreign matter. The sieve used was a cylindrical home swimming pool filter that was 25 cm in diameter and 30 cm long. The filter was made of a plastic grid consisting of 6 mm square openings that covered the sides and bottom of the cylinder. The sieved soil was placed in 1 gallon (3.785 L) plastic Ziploc™ freezer bags and stored in a refrigerator at <4°C until needed for the experiments.

3.2.3 Soil Characterization

An analysis of the soils' physical/chemical characteristics was performed by A & L Great Lakes Laboratories, Inc., located in Fort Wayne, Indiana. This was important as the physical characteristics may influence the biodegradability of aircraft deicing agents in the two soil types. The results of the analyses are

summarized below in Table 3-1. These results confirm that the two soils are different enough to demonstrate potential variations in biodegradation. The complete laboratory report may be found in appendix A.

TABLE 3-1 Analysis of the Soils

Soil	% Sand	% Silt	% Clay	Soil Texture Class	PH	% Organic Matter
Sandy	86	7	7	Loamy Sand	7.35	0.7
High Clay	42	34	24	Loam	8.05	5.25

Method of particle size distribution: MSA Part 1

Source: A & L Great Lakes Laboratories, Inc. Report, Report Number F97220-056, August 12, 1997.

3.2.4 Soil Moisture

The field capacity of the two soils was determined experimentally. A sample from each soil was placed in a plastic cylinder (15 cm long by 2 cm inside diameter). A clean disk of filter paper was taped to one end of the cylinder. The cylinder and filter were weighed empty, and then again with a slightly packed sample of soil. The packed cylinder was placed in a beaker of water so that the filter taped bottom was at least 3 cm under the surface of the water. The cylinder was left in the water for 24 hours and then allowed to drain by gravity for another 2 hours. The cylinder was weighed again and the weight of the cylinder and filter was subtracted. Using the moisture content of the soil, the amount of moisture at maximum field capacity (100%) was determined, along with the amount of moisture needed to bring the two soils up to 70% field capacity.

In order to minimize biodegradation differences in the soils, adjustment of the moisture content to 70% field capacity was used. The 70% level was chosen because it falls in the range of optimal conditions for biodegradation. This level also proved to be convenient in that both of the soils were slightly drier than the 70% field capacity. Adding a specific amount of water to each soil type was much easier than trying to dry the soil to a specific level.

3.3 Microcosm Setup

With the exception of the amount of water and test substance addition, all of the microcosms were prepared in the same way. The microcosms used in this experiment were 250 ml glass bottles. The stainless steel lid on each microcosm had two quick release fittings that allowed plastic tubing to serve as an interface between the microcosm and the respirometer apparatus. The sampled air in the headspace of each microcosm was drawn out through one of the tubes and returned through the other. After each bottle was tared on an Ohaus Harvard Triple Balance, 100 grams of wet soil was added. Once the soil was weighed out, enough water was added to each microcosm to bring the moisture content up to 70% field capacity, and then a measured amount of contaminant was added. See Section 3.6.2 for more details on amounts added. Once all the microcosms were prepared, they were connected to the respirometer and the experiment was begun.

3.4 Respirometer

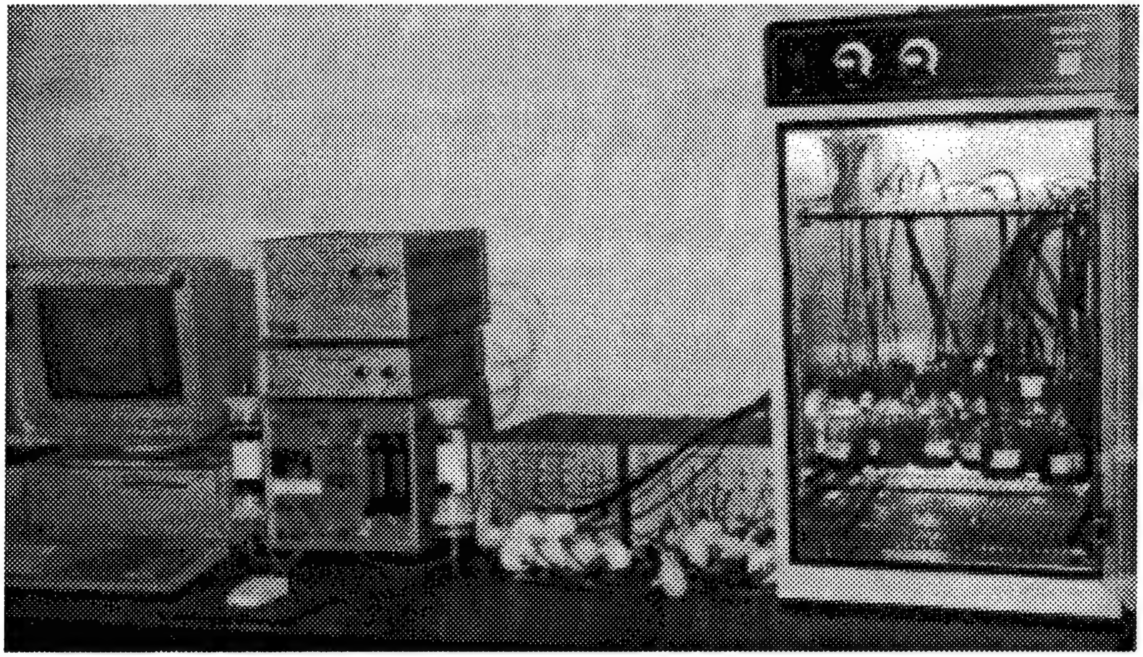
3.4.1 Purpose

This experiment made use of a closed-circuit Micro-Oxymax respirometer, manufactured by Columbus Instruments International Corporation, Columbus, OH. This respirometer was used because of its capability to measure low levels of oxygen consumption and carbon dioxide production resulting from the respiration of the microorganisms in each microcosm. This device also allowed for the measurements to be taken without disturbing the soil microcosms.

3.4.2 Components

The respirometer apparatus consists of the following seven basic components as can be seen from right to left in Figure 3-1 below: an AMBI-HI-LO incubator, manufactured by Lab Line, was used to house, eliminate light, and control the temperature of the 20 microcosms, two expansion interface units were used to direct the flow of the sampled air from each microcosm, a system sample pump controlled the flowrate of the sampled air, an oxygen sensor measured the amount of oxygen in the sampled air, a carbon dioxide sensor measured the amount of carbon dioxide in the sampled air, and a personal computer controlled the experiment and recorded the data.

FIGURE 3-1 Micro-Oxymax Respirometer



3.4.3 Theory

The respirometer circulated air from the headspace of each microcosm through the appropriate expansion unit to the two gas sensors where oxygen or carbon dioxide was measured, and then back to the microcosm in a closed loop configuration. The time between measurements could be varied, and is one of the input parameters when starting an experiment. Measurements were taken every 6 hours, thereby allowing both the rate and cumulative consumption (or production) of oxygen (or carbon dioxide) to be recorded. Each microcosm was refreshed with air after each measurement. Refreshing the microcosms assured that aerobic conditions were being maintained, and that the concentration of gases remained within the detection limits of the sensors. Detection limits for the

two sensors are as follows: oxygen - 19.3%-21.5% and carbon dioxide - 0%-1% (23:2). Through calculations, the amount of degradation of the contaminant was determined by using the gas sensor measurements. Refer to Baker (1995) for a more detailed discussion on the theory and operation of the Micro-Oxymax respirometer.

3.5 Data Collection

The respirometer recorded the amount of oxygen consumed and the amount of carbon dioxide produced every 6 hours. It recorded this information in the form of the following parameters: percent oxygen consumption, percent carbon dioxide production, oxygen consumption rate (μL/min), carbon dioxide production rate (μL/min), cumulative oxygen consumption (μL), cumulative carbon dioxide production (μL). The respirometer also recorded the temperature and the respiratory exchange rate (RER), which is a ratio of carbon dioxide production to oxygen consumption.

3.6 Experiment Setup

3.6.1 Physical

The physical setup of the respirometer was identical for each of the two experiments. The 20 microcosms were kept in the dark, temperature controlled incubator, and were connected to the expansion interface units with 1/8" outside diameter tubing. To prevent moisture from entering the expansion units, filters were attached in line with the tubing. Two 300mL driers, filled with magnesium

perchlorate as the desiccant, were attached to the system sample pump to eliminate any moisture that may have entered the system. The system alternated between the two driers, thereby, allowing the unused one to be changed without stopping the experiment. Another drier filled with Dririte©, also attached to the system sample pump, was used to eliminate moisture from the room air being used to refresh the microcosms after each reading. Because neither PG nor tolyltriazole is volatile, vapor collection was not a concern. Again, figure 3.1 shows the respirometer apparatus.

Using the software package provided, leak and restriction checks were conducted on all the system sensors, microcosms, and tubing prior to the beginning of each experiment. Calibration of the oxygen and carbon dioxide gas sensors was also conducted prior to the experiment being run. This was done by first circulating nitrogen through the sensors to purge them and obtain a zero reading, and then circulating a calibration gas through the system. As stated on the cylinder, the calibration gas, from Liquid Carbonic Company, contained 0.501% carbon dioxide and 20.4% oxygen. The experiment was begun once the calibration and necessary checks, as stated above, were complete.

3.6.2 Statistical

Proving reproducibility of the respirometer was not a major concern since prior studies conducted by John Thomas, Jim Baker, and Chris Totten have all proved that the respirometer is capable of reproducing data between experiments. On

the other hand, repeatability, or the precision of the replicates within the same experiment, was of concern; therefore, it was determined that three replicates of each treatment were the minimum necessary. The total oxygen uptake over time for the different treatments can be compared by averaging and graphing replicates. See Appendix C for these graphs.

As stated in Chapter 1, the objective of experiment 1 was to determine if the biodegradation rate of tolyltriazole was different in the two differing soil types. For this experiment, two milliliters of a 0.25% tolyltriazole in water solution was added to each of the microcosms, but because the dry weight of the two soils in the microcosms was slightly different, the concentrations were also slightly different. The following concentrations resulted: 60 mg/kg for the sandy soil and 65 mg/kg for the high clay soil. Appendix G shows these calculations. The concentration of 0.25% was chosen because 1) tolyltriazole is usually in pure ADAF anywhere from 0.2% to 0.5% (3), and 2) it was a starting point since few, if any, soil biodegradation studies of tolyltriazole have been conducted. The 20 bottles in experiment 1 were split between the two soil types - 10 bottles for the sandy soil, and 10 bottles for the high clay soil. Two of these bottles were run as controls, and contained uncontaminated soil. Experiment 1 was run for 18 days.

The objective of experiment 2 was to determine the affect (inhibition, stimulation, or no effect) of the mixture of tolyltriazole and PG vs. the biodegradation of the contaminants by themselves. For this experiment, only the high clay soil was

used. The high clay soil was chosen because experiment 1 showed that it had a much higher respiration rate (see Figures C-1 and C-2), and was therefore more likely to degrade the contaminants faster. Another reason it was chosen was for its applicability to land treatment situations. A soil with a relatively high clay and high organic content is more likely to be used for land treatment of these wastes than a sandy soil with a low organic content. As stated above, experiment 2 was designed to analyze the effects of a combined mixture of tolyltriazole and PG. Soil was treated with PG alone, tolyltriazole alone and a mixture of both PG and tolyltriazole. Two concentrations of tolyltriazole were used, while the concentration of PG remained constant. Three control bottles with blank soil and two empty bottles were also run so that background respiration could be analyzed. See Table 3-2 below for the physical set up of experiment 2. The concentrations chosen were based on amounts that could be detected by the respirometer over a 2 week period, and on what the soil would typically see in a land treatment system. Experiment 2 was run for 14 days.

TABLE 3-2 Number of Microcosms Used for Each Treatment in Experiment 2

Concentration of Tolyltriazole	Empty	Control	Tolyltriazole Alone	PG Alone	Tolyltriazole/ PG Mix
0 mg/kg	2	3		3	
25 mg/kg			3		3
250 mg/kg			3		3

The concentration of PG was held constant at 1,900 mg/kg.

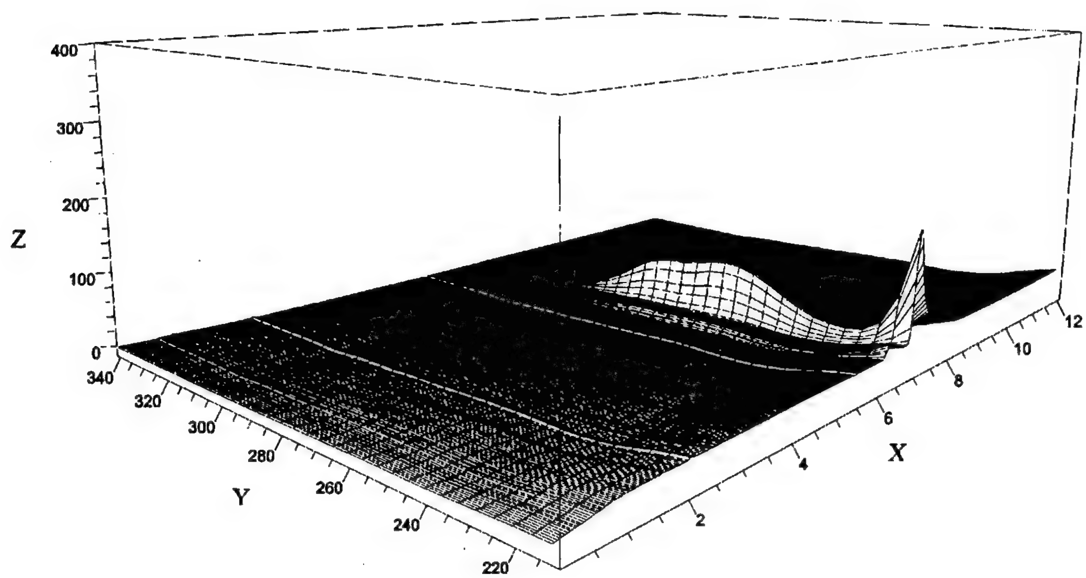
3.7 High Performance Liquid Chromatography (HPLC)

3.7.1 Purpose

Tolyltriazole concentrations were quantified using a Hewlett Packard 1090 Liquid Chromatograph with a Hewlett Packard 1040A diode array detector (DAD). The HPLC was used to determine the amount of tolyltriazole left in the soil samples after the 2 week incubation period in the respirometer. This device was used because of its capability to separate tolyltriazole from other chemicals (soil organics in this case). Because the diode array detector used was unable to detect PG, analysis of PG concentration in the microcosms was not conducted.

The column was an Alltech Adsorbosphere C8 5U 250mm x 4.6mm. The mobile phase consisted of two different solvents; a phosphate buffer composed of 0.5 mL phosphoric acid (H_3PO_4) and 0.65 g potassium dihydrogen phosphate (KH_2PO_4) in water, and HPLC grade methanol. The solvents were set up in a ratio and gradient that allowed for the tolyltriazole to peak at a reasonable time (roughly 8 min) and then flush the column of any soil organics. The solvent ratio started at 30:70 buffer:methanol and gradually moved to 50:50 buffer:methanol in the first 10 min. At the 10 min mark, the ratio jumped to 10:90 buffer:methanol and stayed constant for the next 15 min. The above method use to detect tolyltriazole was modified from a method provided by PMC Specialties Group, Inc. of Cincinnati, OH. All of the sample injection volumes were 10 μL, and the tolyltriazole was detected at a wavelength of 280 $\pm$ 2 nm. Figure 3-2 below shows a 3-D picture of the tolyltriazole peak.

FIGURE 3-2 3-D Tolyltriazole Peak



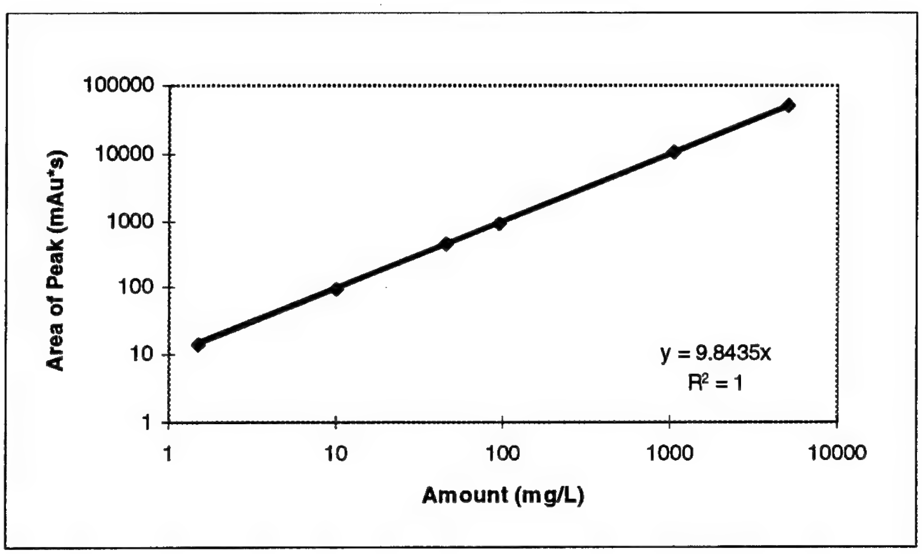
X = time (min)
Y = wavelength (nm)
Z = absorbance (microabsorbency units (μAu))

3.7.2 Quantitative Tolyltriazole Analysis

Known concentrations of tolyltriazole were run through the HPLC to create a calibration curve (see Figure 3-3 below). This curve was used to quantify the amount of tolyltriazole left in the soil samples upon completion of the two week respirometer experiment. Once each respirometer experiment was complete, samples from each soil type were taken from the microcosms and placed in 40 mL glass bottles. Roughly 15 mL of methanol was added to each bottle in order to extract any tolyltriazole from the soil particles. Each bottle was weighed three times: empty, with the soil sample, and with the soil and the methanol. The 40

mL bottles were rotated on a Glas-Col Laboratory Rotator for 24 hours and then centrifuged for 15 min at a speed of 1000 rpm in a centrifuge manufactured by Fisher Scientific (Marathon 12KBR). After being centrifuged, liquid samples were extracted using a syringe and a 0.45μm Gelman Sciences Acrodisc syringe filter, and placed into the HPLC for analysis. Comparing this data to the calibration curve below, the concentration of tolyltriazole left in the soil could be determined.

FIGURE 3-3 Calibration Curve for HPLC Results



IV. Data Analysis

4.1 Overview

Among the techniques used to analyze the data from both the respirometer and the HPLC were graphical comparisons, and both descriptive and analytical statistics. The data from the respirometer was used to determine the biological activity of the microorganisms in the soil. For experiment 1, this activity was compared to determine whether or not there was a difference in oxygen consumption rate between the two soil types. For experiment 2, the activity was compared for the uncontaminated, tolyltriazole contaminated, PG contaminated, and PG/tolyltriazole contaminated high clay soil. This data was used to determine the effect, if any, of tolyltriazole and PG alone and when acting together on their biodegradation rates. In order to make conclusions regarding the results, statistical hypotheses were tested for each experiment.

4.2 Soil Type Differentiation

Experiment 1 was conducted to determine whether or not there was a difference in the oxygen consumption of the microorganisms when contaminated with tolyltriazole in the two soil types. This was tested using the ANOVA and Tukey pairwise comparison of means tests. The ANOVA was used to determine whether or not the level of tolyltriazole and soil type interact. In order for it to be proven that interaction was taking place, the high clay soil should have produced proportionately higher levels of oxygen consumption at the higher level of contamination than the sandy soil. However, it was found, for the two levels of

tolyltriazole used (0 and 65 mg/kg for high clay and 60 mg/kg for sandy soil), the contaminant level and soil type did not interact to affect oxygen consumption. Both Figures C-1 and C-2 show that the O_2 consumption in the contaminated soil closely followed the uncontaminated soil for both the high clay and the sandy soil types. Figure C-1 depicts the oxygen consumption rate of the treatments, while Figure C-2 shows the cumulative oxygen consumption. Reasons for the above results may be that 65 mg/kg and 60 mg/kg of tolyltriazole was not enough to make a detectable difference in the overall oxygen consumption of the microorganisms. Because very few biodegradation studies have been conducted on tolyltriazole, these levels were chosen as starting points. Table 4.1 gives the results of the ANOVA test on the experimental data. Details of the ANOVA test can be found in Appendix B.

TABLE 4-1 Results of ANOVA on Factors Fuel and Soil Type

H_o: (Null Hypothesis)	F Statistic for Test	F Statistic for Rejection	Decision
Factors do not interact to affect oxygen consumption	MS(AB)/MSE=0.0184	If $F_{statistic}>F_{0.05,1,12}=4.75$	Accept H_o

The Tukey pairwise comparison of means test was used to determine whether or not there was a significant difference in oxygen consumption between the two soil types. Table 4.2 shows the results of the comparison at the two different fuel levels. There is, in fact, a significant difference in oxygen consumption between the two soil types. Details of the Tukey test can be found in Appendix B.

TABLE 4-2 Tukey Pairwise Comarison of Means by the Factor Fuel

Pair	Difference	Half CI	Sig Diff?
0 mg/kg, S vs HC	122,908	57,314	Yes
60 & 65 mg/kg, S vs HC	159,431	57,314	Yes

Again, Figures C-1 and C-2 support this analysis.

4.3 Tolyltriazole and PG Biodegradation

Experiment 2 was conducted to determine the affect (inhibition, stimulation, or no effect) of the mixture of tolyltriazole and PG vs. the biodegradation of the contaminants by themselves. The null hypotheses used for this experiment was that there would be no effect. Biodegradation could be concluded, provided that the difference in the sample means of oxygen uptake for contaminated soil and uncontaminated soil is significantly larger than the null hypothesis distribution, which is centered around zero. To determine where biodegradation, inhibition, or no effect occurred, a two tailed t test with a level of confidence of 95% was performed at each sampling interval. A summary of the sample data for the individual contaminants can be found in Tables D-1, D-2, and D-3 in Appendix D, and for the combined contaminants in Tables E-1 and E-2 in Appendix E.

Figure D-1, which shows the 95% confidence interval of the difference of the means for the soil contaminated with 25 mg/kg tolyltriazole, verifies the results of the two tailed t test found in Table D-1. Because the confidence interval hooks zero (where the null is centered) at each interval, it can be concluded that this

level of contamination had no effect on the oxygen consumption of the microorganisms. Although this result was not surprising, based on the fact that no effect was seen in experiment 1 with the addition of 65 mg/kg tolyltriazole, the treatment was needed in order to make a comparison with the combined PG/tolyltriazole treatment. From Table D-2 and Figure D-2, it can be seen that biodegradation of 250 mg/kg tolyltriazole occurred after a lag time of roughly 4.5 days, and continued throughout the remainder of the experiment. Table D-3 and Figure D-3 show that biodegradation of 1,900 mg/kg PG occurred immediately and then reduced to background levels after only 36 hrs. Figure E-1 and Table E-1 show that for the treatment of 25mg/kg tolyltriazole and 1,900 mg/kg PG, measurable biodegradation occurred after a lag time of roughly 1 day and continued again throughout the remainder of the experiment. Figure E-2 and Table E-2 correspond to the combined treatment of 250 mg/kg tolyltriazole and 1,900 mg/kg PG. Once again, biodegradation was detectable after only 12 hours and continued throughout the remainder of the experiment. The data in Appendix E indicates that the combination of the two contaminants increases biodegradation over the sum of their individual components; however, it is impossible from this data to determine how the interactions of the two contaminants caused this increase in oxygen consumption. One possible answer is that tolyltriazole is acting as a surfactant and making the PG more readily available to the microorganisms.

All the oxygen consumption curves (cumulative and rate) can be seen in Appendix C. From these figures, it can be seen that the combined contaminants had a much greater impact on the oxygen consumption than the individual contaminants. One oddity that was noticed was that the curve for the combined 250 mg/kg tolyltriazole and 1,900 mg/kg PG seemed to peak and then plateau for about 4 days. It was determined, however, after looking back at the original data, that the percent oxygen consumption readings for this treatment were less than the allowable range of 19.3-21.5 for the oxygen sensor. This limitation would explain the plateau in the curve at those points.

The mean oxygen consumption curves and confidence intervals for the different treatments vs. the uncontaminated soil are shown in Appendix F. Figures F-1, F-2, and F-3, which depict the contaminants individually, show the confidence intervals overlapping one another. This overlap indicates that there is not a significant difference between oxygen consumption of the uncontaminated and contaminated soils. Figures F-4 and F-5 , which depict the combined contaminants, show that there is a significant difference in oxygen consumption between the contaminated and the uncontaminated soils since their confidence intervals do not overlap. This again indicates that the combination of the two contaminants increases the biodegradation; though, again it is impossible to conclude from this data the mechanism causing the increase in oxygen consumption.

4.4 HPLC Results

In order to measure the amount of tolyltriazole left in the microcosms upon completion of the respirometer experiments, samples of soil were taken from the microcosms and analyzed with an HPLC. Four microcosms from each of the two soil types were randomly chosen from experiment 1. The average percent of tolyltriazole recovered from the high clay and sandy soils was 36% and 40% respectively. For experiment 2, samples from all of the microcosms containing tolyltriazole were run through the HPLC. Table 4.3 below gives average percent recovered from each of the treatments containing tolyltriazole.

TABLE 4-3 HPLC Results for Experiment 2

Concentration (mg/kg)	Average % Tolyltriazole Recovered
25 Tolyltriazole	11.5
250 Tolyltriazole	64
25 Tolyltriazole/1,900 PG	11.5
250 Tolyltriazole/1,900 PG	55

Because one of the microcosms containing 25 mg/kg tolyltriazole alone gave a reading of 97% recovery, it was considered an anomaly and dropped from the average.

The removal efficiency of tolyltriazole from the two soil types was determined by taking two samples from each type of freshly contaminated soil and running them through the HPLC. The two microcosms containing high clay soil were

contaminated with 250 mg/kg tolyltriazole, while the two containing sandy soil were contaminated with 120 mg/kg tolyltriazole. The microcosms were allowed to sit for 2 hours before the tolyltriazole was extracted. The extraction procedure followed was the same as that for experiments one and two, which is explained in Chapter Three. The removal efficiency for the sandy soil was found to be around 85% while that for the high clay soil was around 90%. Again, one of the bottles for the high clay soil was dropped and considered an anomaly since it gave a removal efficiency reading of 126%. The raw data and calculations for the HPLC results can be seen in Appendix H.

The above results indicate that it is possible to recover and detect roughly 87% of the tolyltriazole when loaded on soil. Based on the recovery efficiency and the results from experiments 1 and 2, it can be concluded that biodegradation occurred in the microcosms, and the addition of PG did not make a difference on the overall degradation of tolyltriazole.

The HPLC results indicate that biodegradation of tolyltriazole occurred in all the contaminated microcosms, while the oxygen consumption curves (Appendix B) and the two sample t test (Appendix D) indicate that no biodegradation occurred in the microcosms contaminated with 25 mg/kg tolyltriazole alone. This contradiction is most likely the result of 25 mg/kg tolyltriazole not being a high enough concentration to stimulate a detectable increase in microbial respiration above background levels.

4.5 O_2/CO_2 Ratio Comparisons

Along with the amount of O_2 being consumed, the amount of CO_2 being produced is also a measure of the biodegradation. Provided that there are adequate amounts of nutrients in the soil, both the O_2 consumption and CO_2 production by the microorganisms depend on the amount of carbon source (substrate) present. However, O_2 consumption is a more accurate measurement since portions of the carbon can be transformed into intermediate products and converted to cell biomass rather than being released as CO_2. Therefore, the amount of CO_2 that is being produced may not be an accurate measure of the biodegradation rate. The ratio of O_2 consumption to CO_2 production can, however, be a good estimate of the amount of carbon that is trapped in the soil system, and therefore, how much substrate has been transformed.

An increase in the O_2/ CO_2 is thought to indicate an increase in the transformation of substrate into intermediate products and cell biomass. It is also suspected that this ratio increases as a result of an increase in substrate available to the microorgansism. The additional carbon source stimulates the growth of microorganisms, thus increasing the amount of O_2 being consumed and thereby leading to an increase in the O_2/ CO_2 ratio. Based on this postulate, the increase in the ratio is further proof that biodegradation is occurring.

The O_2/ CO_2 ratios for each treatment in experiment 2 were calculated and compared. Table H-1 and figure H-1 show these results. From Figure H-1, it

can be seen that there is a noticeable difference among some of the treatments. All of the microcosms which were contaminated with a single contaminant (tolyltriazole or PG alone), do not differ much, and show the same pattern as the control microcosms. The microcosms which had a combination of tolyltriazole and PG show a noticeable difference and a different pattern. These results agree with the other statistical tests and confirm that the highest rate of biodegradation was occurring in the microcosms contaminated with 250 mg/kg tolyltriazole and 1,900 mg/kg PG, followed by the one contaminated with 25 mg/kg tolyltriazole and 1,900 mg/kg PG, and then the ones contaminated with the individual contaminants.

V. Conclusions and Recommendations

5.1 Conclusions

Previous studies on the biodegradation of PG in soil have determined that it is readily degradable; however, the biodegradation of many of the other components of ADAFs, such as tolyltriazole, have not been studied. The purpose of this thesis was to determine the biodegradability of tolyltriazole both by itself and when combined with PG. A respirometer was used in this experiment to measure the amount of oxygen consumption for each of the microcosm treatments. Oxygen consumption curves and statistical tests were used to determine whether or not biodegradation was occurring.

Repeatability of the respirometer was proven by the fact that the oxygen consumption curves for the replicates of each treatment in each experiment were consistent with one another. Reproducibility of the respirometer was not a concern in this experiment since it has been proven in previous experiments conducted by Thomas (1996), Totton (1995), and Baker (1995).

Experiment 1 tested tolyltriazole alone in two different soil types, a sandy soil and a high clay soil. This experiment proved that there was a difference in oxygen consumption between the soil types, with the high clay soil being higher. This conclusion would imply that biodegradation would occur at a greater rate in the high clay soil than in the sandy soil, and would be more applicable for land

treatment. The results from experiment 1 also indicated that the oxygen consumption for a given soil type stayed the same whether tolyltriazole was added to the soil or not. However, it was determined through theoretical oxygen demand calculations that these levels of tolyltriazole were below the detection limits of the respirometer.

Experiment 2 was set up based on the results from experiment 1. Only the high clay soil was used, and two different levels (25 mg/kg and 250 mg/kg) of tolyltriazole and one level of PG (1,900 mg/kg) were used. It was again found that when the tolyltriazole alone was added to the soil, respiration did not differ much from the control, even at the higher level. When the two tailed t-test was conducted on the lower of the two concentrations of tolyltriazole, it suggested that there was no biodegradation occurring; however, the results from the HPLC indicated that biodegradation did occur. This difference in results confirms the idea that the concentration of tolyltriazole that was added to the soil was not enough to significantly increase the oxygen consumption of the microorganisms. The results from the two tailed t-test for the higher of the two tolyltriazole levels were consistent with those of the HPLC which indicated that biodegradation was occurring; however, the two tailed t-test indicated that there was a lag period of roughly 4.5 days. Also, the 95% confidence interval oxygen consumption curves (Appendix F) do not show a significant difference between the 250 mg/kg amount and the control.

The amount of 1,900 mg/kg PG was chosen based on the amounts used in previous experiments conducted by Lt Halterman-O'Malley on the basis that it would degrade within a 2 week period. The two tailed t-test shows biodegradation began almost immediately and then ended after only 36 hours, implying that it all degraded. However, because the respirometer readings for the three PG contaminated microcosms had a fairly large standard deviation, I would conclude that biodegradation continued longer than 36 hours. Efforts to use the HPLC to analyze the soil for PG proved unsuccessful, therefore, the respirometer was the only device used to measure the biodegradation of PG.

The two levels of tolyltriazole were combined with the one level of PG. Once again, both the two tailed t-test and the HPLC results indicate biodegradation; however the oxygen consumption curves seem to indicate an effect which is more than additive, and the 95% confidence interval oxygen consumption curves show that there is a significant difference between these combinations and the control. The reason for this drastic increase in oxygen consumption is not known; however, because the amount of tolyltriazole that was recovered in both the tolyltriazole alone and combined mixture treatments was the consistent, it can be speculated that the increase in respiration was from the increased biodegradation of PG. It is possible that the tolyltriazole is enhancing the biodegradation of the PG by making it more available to the microorganisms.

The O_2/CO_2 ratio is also an indication of biodegradation since an increase in the ratio is thought to indicate an increase in the transformation of substrate to cell mass and intermediate compounds. The ratios calculated for each treatment in experiment 2 confirm the conclusion that biodegradation was occurring and agreed with the results found in the other statistical tests. The following are the treatments in decreasing rate of biodegradation from highest to lowest: the combined treatment of 250 mg/kg of tolyltriazole and 1,900 mg/kg PG, the combined treatment of 25 mg/kg of tolyltriazole and 1,900 mg/kg PG, the treatment of 1,900 mg/kg PG alone, the treatment of 250 mg/kg of tolyltriazole alone, and the treatment of 25 mg/kg tolyltriazole alone.

5.2 Improvements

The results from this experiment could have been improved by analyzing the amount of PG left in the soil through the use of a gas chromatograph (GC). This would help to determine whether or not the tolyltriazole was having an affect on the biodegradation of the PG.

Sorption isotherm tests done on tolyltriazole would also help to the results. Because it is not known exactly how tolyltriazole sorbs to the soil, it is difficult to tell whether or not it is readily available to the microorganisms.

5.3 Follow-On Research

There are five recommendations for possible follow-on research that can be conducted; sorption isotherm tests on tolyltriazole using GC analytical methods to determine concentrations of PG in the soil, analyzing a different component of ADAFs, recontaminating the soil with tolyltriazole or another component of ADAFs, and adding surfactants or nutrients to the soil.

5.3.1 Sorption Isotherms

Sorption isotherms for tolyltriazole and soil can be constructed with the use of the HPLC. These isotherms will help in understanding the availability of the tolyltriazole to the microorganisms, and ultimately in its biodegradability.

5.3.2 GC Analysis

The use of the GC will help to determine the amount of PG left in the soil after the respirometer experiment. This will also help to determine whether or not the tolyltriazole is enhancing the biodegradation of the PG.

5.3.3 Analyzing Other Components of ADAFs

The biodegradation of other components of ADAFs such as wetting agents, surfactants, and thickeners can also be studied. The study of how they degrade by themselves and when mixed with each other is of importance to the overall biodegradation of the ADAF.

5.3.4 Recontamination of the Soil

Recontamination of the soil with tolyltriazole, a mixture of tolyltriazole and PG, or another component of the ADAF will help to determine the overall applicability of a land treatment system, since the soil would be seeing the contaminant more than once. The reapplication will help to determine whether or not microorganisms will acclimate to the contaminant and begin to degrade in faster.

5.3.5 Surfactant or Nutrient Addition

The addition of surfactants or nutrients to the soil would help to determine whether or not they have an effect on the biodegradation rates of tolyltriazole, PG, or a combination of the two. It would also help to determine which of the two contaminants is causing the increase in oxygen consumption when the two are combined.

5.3.6 Soil Properties Study

Determining which of the soils properties (clay content, pH, organic content, etc) play the biggest role in the biodegradation process would help to determine what type of soil would be best for a land treatment system.

APPENDIX A SOIL CHARACTERIZATION REPORT

REPORT NUMBER: F97220-056
ACCOUNT NUMBER: 96600

A & L GREAT LAKES LABORATORIES, INC.

3505 Conestoga Drive • Fort Wayne, Indiana 46808-4413 • Phone (219)483-4759 • FAX (219)483-5274

REPORT OF ANALYSIS

TO: AFIT/ENV
2950 P STREET
WRIGHT-PATTERSON AFB, OH 4543

DATE RECEIVED: 8/8/97
DATE REPORTED: 8/12/97
PAGE: 1
P.O. NUMBER: F33600-97-M-0460

ATTN: CAPT LAURA JOHNSON

RE: PR # F61TNV71690100

LAB NO.	SAMPLE ID	ANALYSIS	RESULT	UNIT	METHOD
7188	1A	Sand	43	%	MSA Part 1 (1986) pp 404-408
		Silt	34	%	MSA Part 1 (1986) pp 404-408
		Clay	23	%	MSA Part 1 (1986) pp 404-408
		Soil Textural Class	Loam		MSA Part 1 (1986) pp 383-385
7189	1B	Sand	85	%	MSA Part 1 (1986) pp 404-408
		Silt	8	%	MSA Part 1 (1986) pp 404-408
		Clay	7	%	MSA Part 1 (1986) pp 404-408
		Soil Textural Class	Loamy Sand		MSA Part 1 (1986) pp 383-385
7190	1C	Sand	87	%	MSA Part 1 (1986) pp 404-408
		Silt	6	%	MSA Part 1 (1986) pp 404-408
		Clay	7	%	MSA Part 1 (1986) pp 404-408
		Soil Textural Class	Loamy Sand		MSA Part 1 (1986) pp 383-385
7191	1D	Sand	41	%	MSA Part 1 (1986) pp 404-408
		Silt	34	%	MSA Part 1 (1986) pp 404-408
		Clay	25	%	MSA Part 1 (1986) pp 404-408
		Soil Textural Class	Loam		MSA Part 1 (1986) pp 383-385

Report Number: F97220-056
Account Number: 96600

A & L GREAT LAKES LABORATORIES, INC.

3505 Conestoga Drive • Fort Wayne, Indiana 46808-4413 • Phone 219-483-4759 • Fax 219-483-5274

To: AFIT/ENV
2950 P STREET
WRIGHT-PATTERSON AFB, OH 4543

For: PR # F61TNV71690100

Attn: CAPT LAURA JOHNSON

Date Received: 8/8/97 Date Reported: 8/12/97

P.O. Number: F33600-97-M-0460

SOIL TEST REPORT

SAMPLE NUMBER	LAB NUMBER	ORGANIC MATTER %	PHOSPHORUS BRAY P1 ppm-P	PHOSPHORUS BRAY P2 ppm-P	POTASSIUM K ppm	MAGNESIUM Mg ppm	CALCIUM Ca ppm	SODIUM Na ppm	pH SOIL pH	pH BUFFER pH	Cation Exchange Capacity meq/100g	PERCENT BASE SATURATION % K	% Mg	% Ca	% H	% Na
1A	7188	5.1	10 *L*		106 *M*	300 *H*	2100 *H*		7.3		13.3	2.0	18.8	79.1		
1B	7189	0.8	1 *VL*		24 *VL*	130 *VL*	5050 *VH*		8.1		26.4	0.2	4.1	95.7		
1C	7190	0.6	2 *VL*		26 *VL*	135 *VL*	5300 *VH*		8.0		27.7	0.2	4.1	95.7		
1D	7191	5.4	9 *VL*		104 *M*	295 *H*	2200 *H*		7.4		13.7	1.9	17.9	80.1		

VL = VERY LOW *L* = LOW *M* = MEDIUM *H* = HIGH *VH* = VERY HIGH

SAMPLE NUMBER	SULFUR S ppm	ZINC Zn ppm	MANGANESE Mn ppm	IRON Fe ppm	COPPER Cu ppm	BORON B ppm	SOLUBLE SALTS mmhos/cm	NITRATE NO_3-N ppm	AMMONIUM NH_4-N ppm	BICARB-P P ppm		COMMENTS

APPENDIX B ANOVA TESTS FOR FUEL AND SOIL TYPE VS. O_2 CONSUMPTION

Analysis of Variance Table for Cumulative O_2 (CUMO2).

SOURCE	DF	SS	MS	F	P
TOLY_LVL (A)	1	1.792E+09	1.792E+09	2.41	0.1468
SOIL (B)	1	7.972E+10	7.972E+10	107.02	0.0000
A*B	1	1.3340E+09	1.334E+09	1.79	0.2056
RESIDUAL	12	8.938E+09	7.449E+08		
TOTAL	15	9.178E+10			

TEST (α=0.5)
H_o: Tolyltriazole level and soil type do not interact to affect O_2 consumption.
H_a: Tolyltriazole level and soil type do interact to affect O_2 consumption.

Rejection Region: $F > F_{\alpha,v1,v2}$

Mean Square of Interaction, MS(AB) = 1.370E+07
Mean Square of Error, MSE = 7.449E+08

F Statistic for Interaction = MS(AB)/MSE = 0.0184
$F_{\alpha,v1,v2} = F_{0.05,\ 1,12} = 4.75$

0.0184 < 4.75, therefore accept H_o, factors do not interact to affect the O_2 consumption.

RAW DATA: MEANS OF CUMULATIVE O_2 (μL) FROM EACH TREATMENT

TOLY_LVL: 1 = 0 mg/kg, 2 = 60 mg/kg for sand and 65 mg/kg for high clay
SOIL: 1 = Sandy, 2 = High Clay
REPLICATION: 1, 2, 3, 4

CASE	$CUMO_2$	TOLY_LVL	REPLICATE	SOIL
1	47586	2	1	1
2	47483	2	2	1
3	46192	2	3	1
4	39376	2	4	1
5	54344	1	1	1
6	36589	1	2	1
7	35831	1	3	1
8	42254	1	4	1
9	239995	2	1	2
10	200384	2	2	2
11	175911	2	3	2
12	202072	2	4	2
13	209296	1	1	2
14	172864	1	2	2
15	99104	1	3	2
16	179385	1	4	2

TUKEY PAIRWISE COMPARISON OF THE MEANS

Level of Significance	α=0.05
Levels of factor a (soil types)	a=2
Levels of factor b (toly level)	b=2
Number of Replications	n-4
MSE from ANOVA	MSE=7.449E+08
Varience of D_{hat} (2MSE/n)	s^2=3.72E+8
Std Deviation of D_{hat}	s=19,299

Difference between means $D=\mu_{ij}-\mu_{i'j'}$

The Tukey multiple

From Table A.8 (pg711 of Devore), the student's t:
q(0.05, 4, 12) = 4.2

Plug the student's t into the Tukey Multiple:
$T=[1/(2)^{1/2}]*q_{0.05,4,12} = 2.9698$

Confidence Interval **95%CI=±T*s = ±57,314**

Mean Cumulative O2 (μl)		
Factors	**Tolyltriazole Level**	
Soil/Fuel	0 mg/kg	60 & 65 mg/kg (s & hc)
Sandy	42,254	45,159
High Clay	165,162	204,590

If the difference between the pairs is greater than one half the confidence interval, then there is a significant difference between the pairs.

Pair	Difference	Half CI	Sig Diff?
0 mg/kg, S vs HC	122,908	57,314	Yes
60 & 65 mg/kg, S vs HC	159,431	57,314	Yes

APPENDIX C RESPIROMETER OXYGEN CONSUMPTION CURVES FOR EACH TREATMENT

The oxygen consumption curves for both experiments can be seen on the following pages. The amount of oxygen consumed was used to estimate the amount of biodegradation. Except for being smaller in scale, the carbon dioxide production graphs are identical to the oxygen consumption graphs, and therefore are not shown.

<u>LIST OF FIGURES</u>

FIGURE C-1 Experiment 1- Mean O_2 Consumption Rate for 65 mg/kg Tolyltriazole in High Clay Soil vs. 60 mg/kg Tolyltriazole in Sandy Soil vs. the Controls

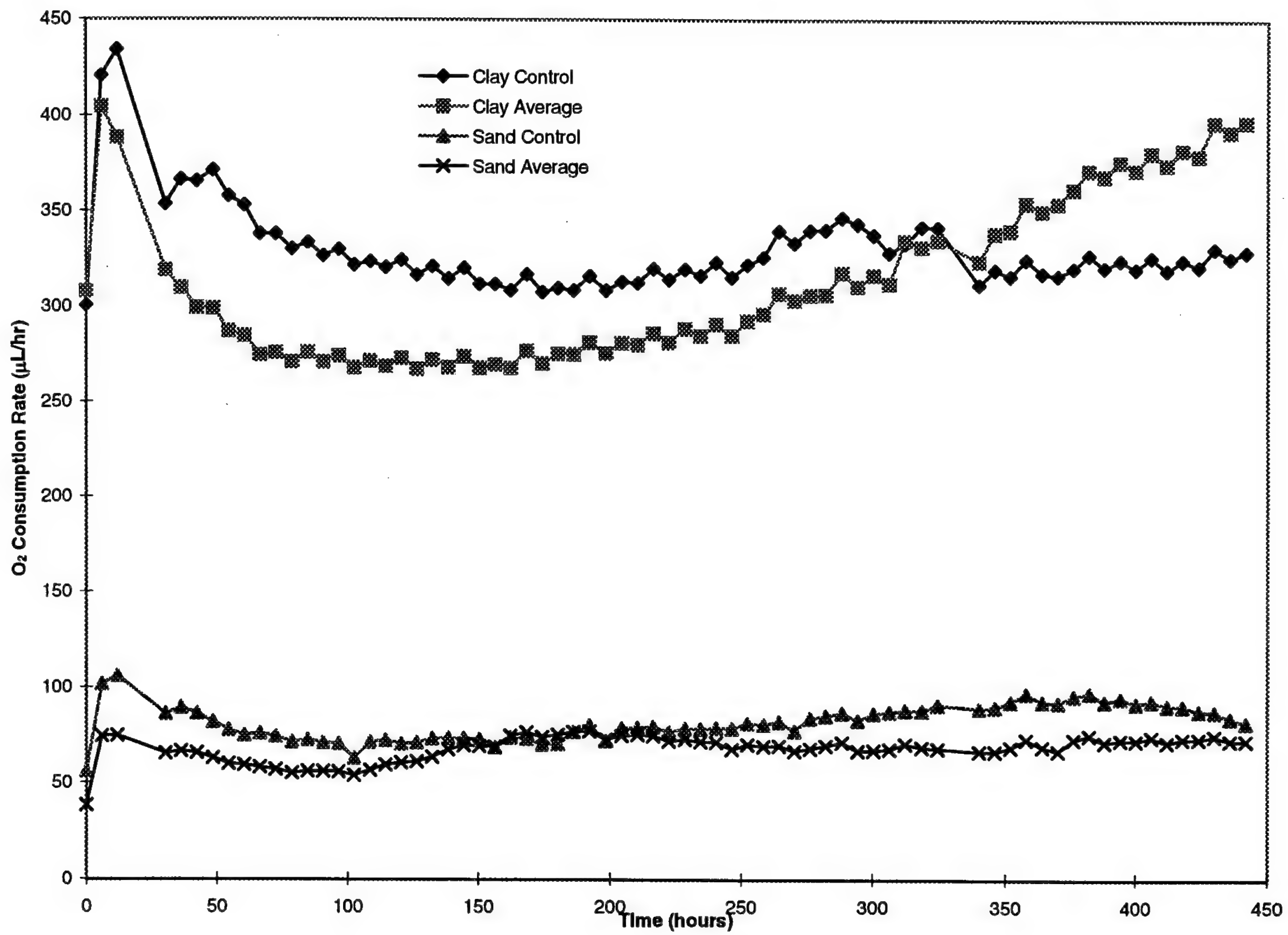


FIGURE C-2 Experiment 1- Mean Cumulative O_2 Consumption and Standard Error for 65 mg/kg Tolyltriazole in High Clay Soil vs. 60 mg/kg Tolyltriazole in Sandy Soil vs. the Controls

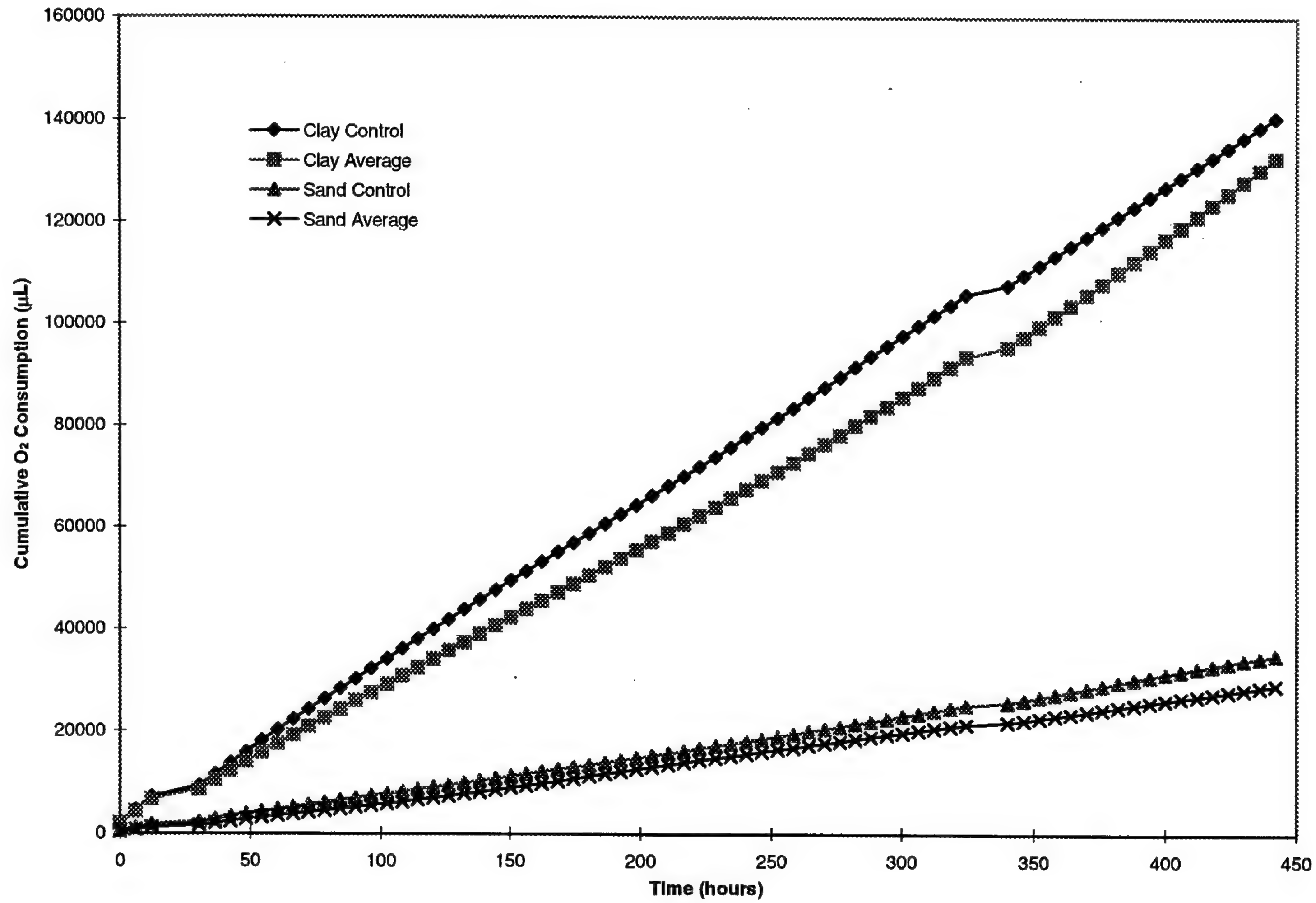


FIGURE C-3 Experiment 2 - Mean O_2 Consumption Rate For All Treatments In High Clay Soil

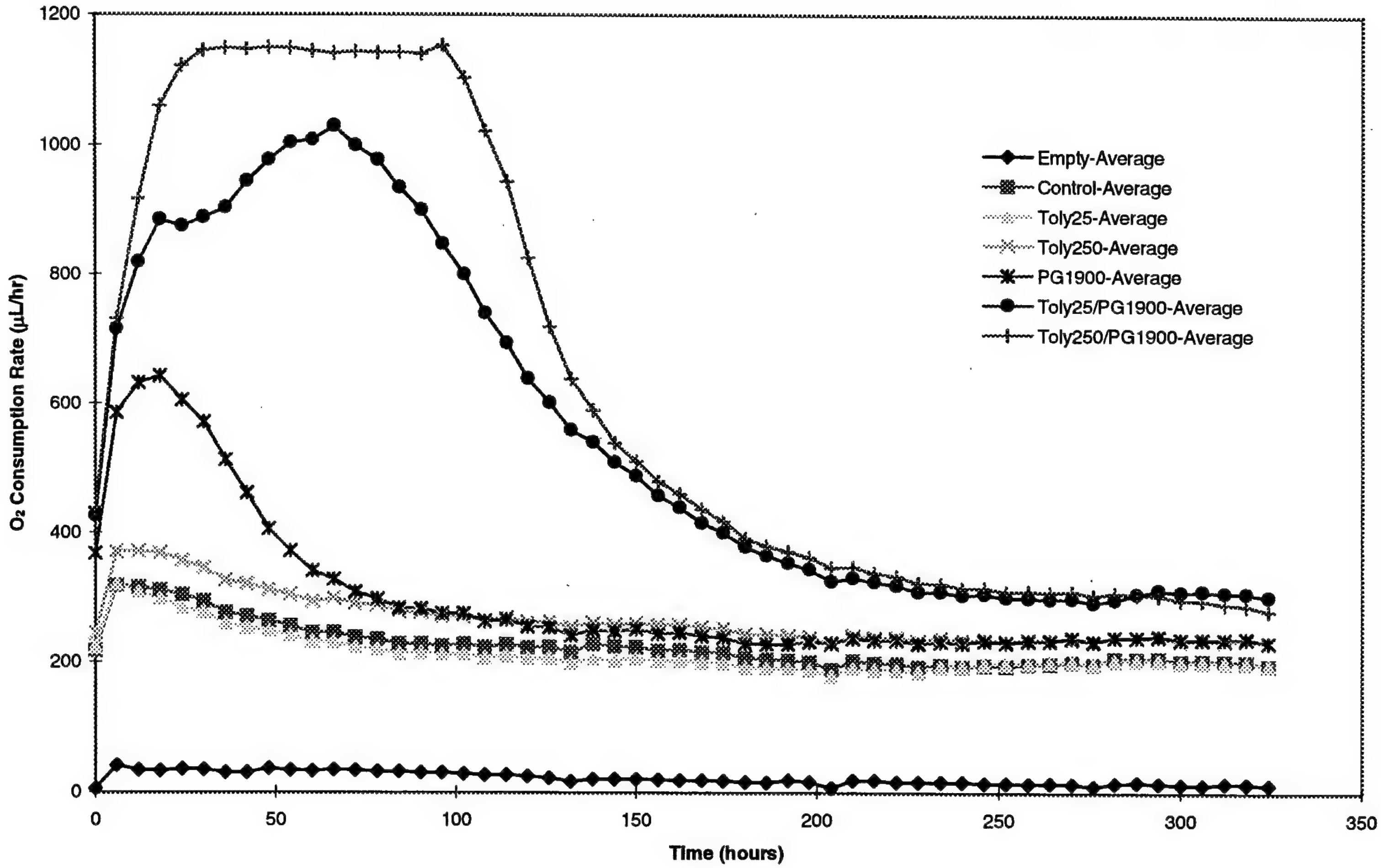


FIGURE C-4 Experiment 2 - Mean O_2 Consumption Rate for 25 mg/kg Tolyltriazole vs. 250 mg/kg Tolyltriazole vs. 1,900 mg/kg PG vs. the Control - All in High Clay Soil

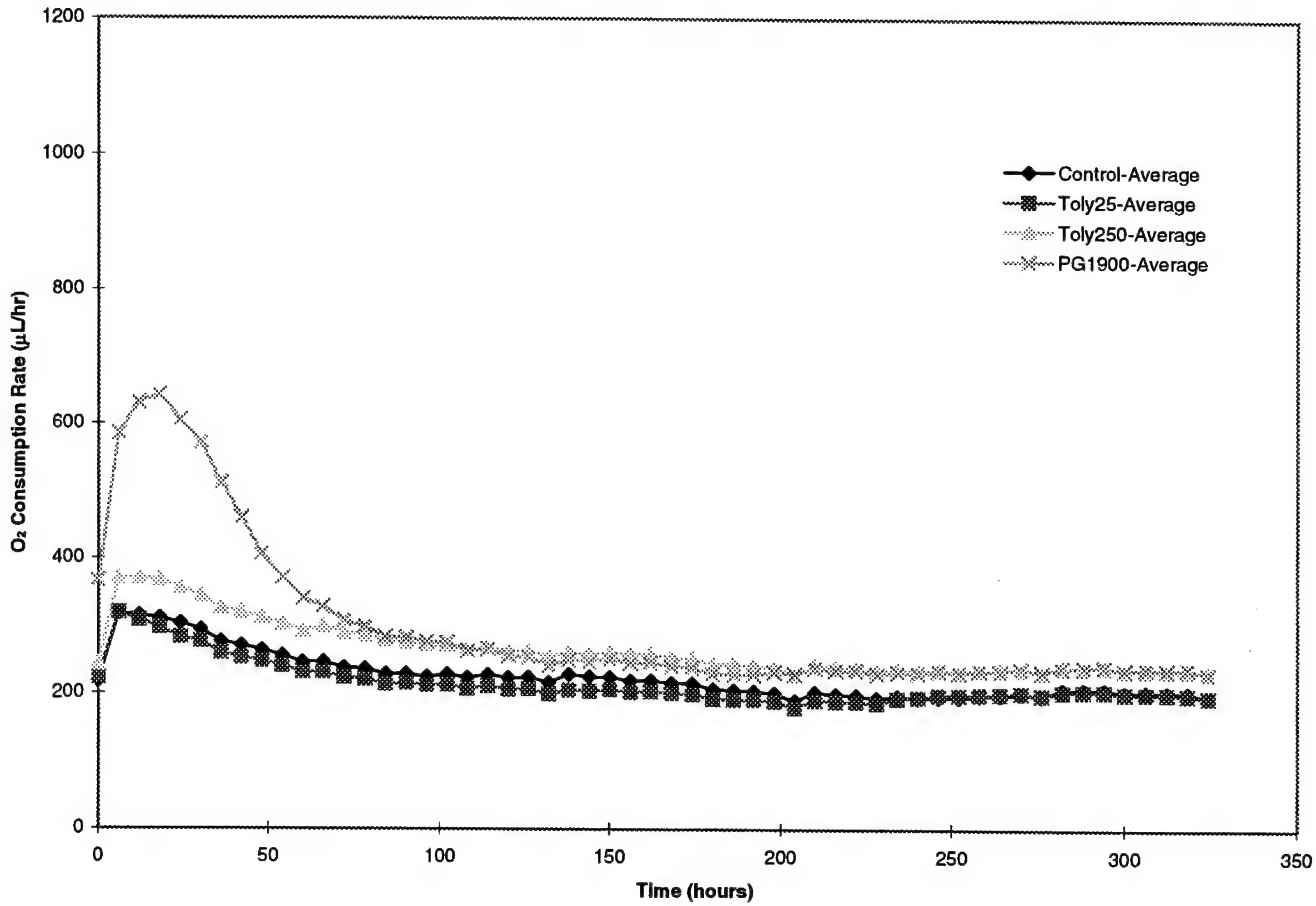


FIGURE C-5 Experiment 2 - Mean O_2 Consumption Rate for 25 mg/kg Tolyltriazole vs. 25 mg/kg Tolyltriazole/1,900 mg/kg PG vs. 1,900 mg/kg PG vs. the Control - All in High Clay Soil

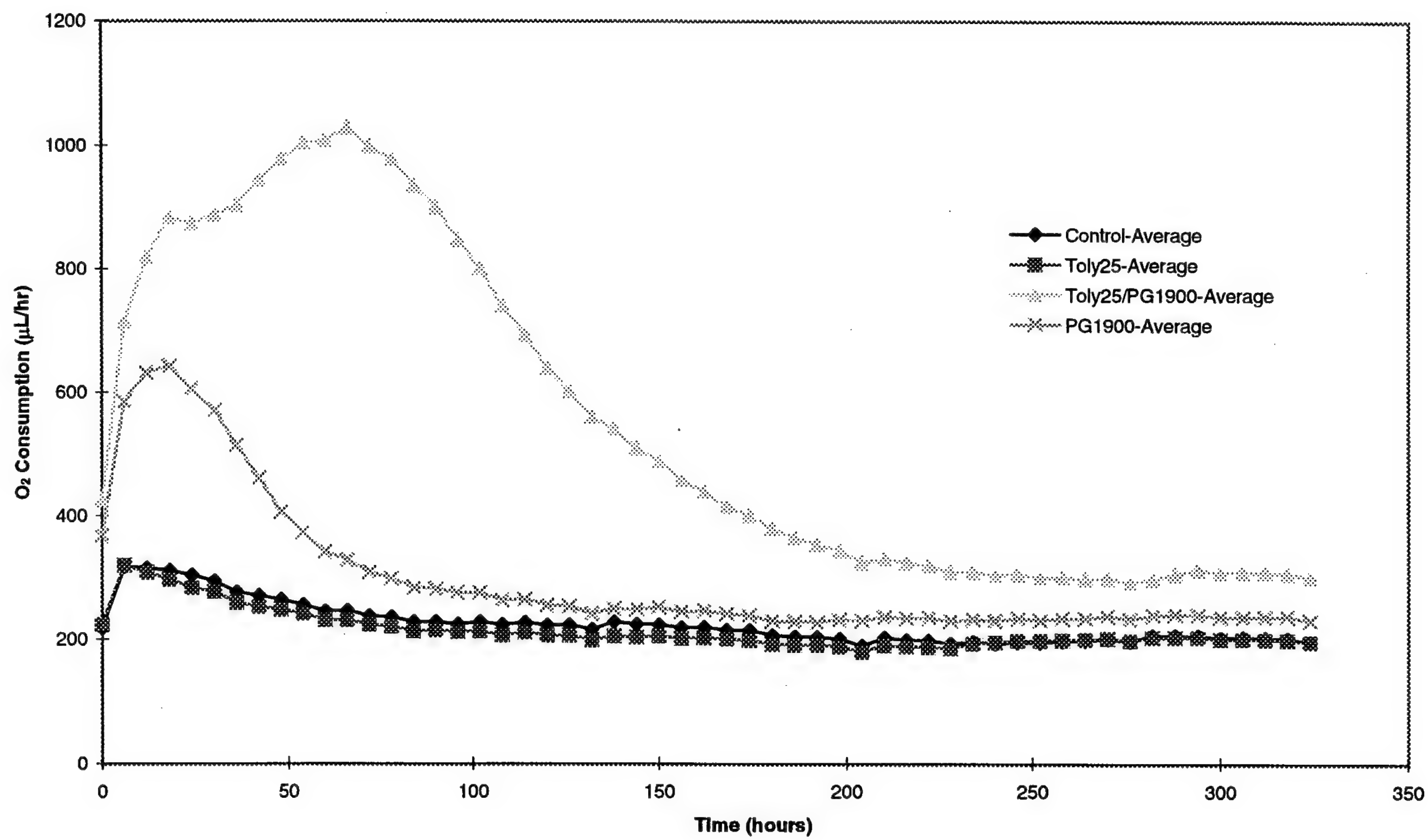


FIGURE C-6 Experiment 2 - Mean O_2 Consumption Rate for 250 mg/kg Tolyltriazole vs. 250 mg/kg Tolyltriazole/1,900 mg/kg PG vs. 1,900 mg/kg PG vs. the Control - All in High Clay Soil

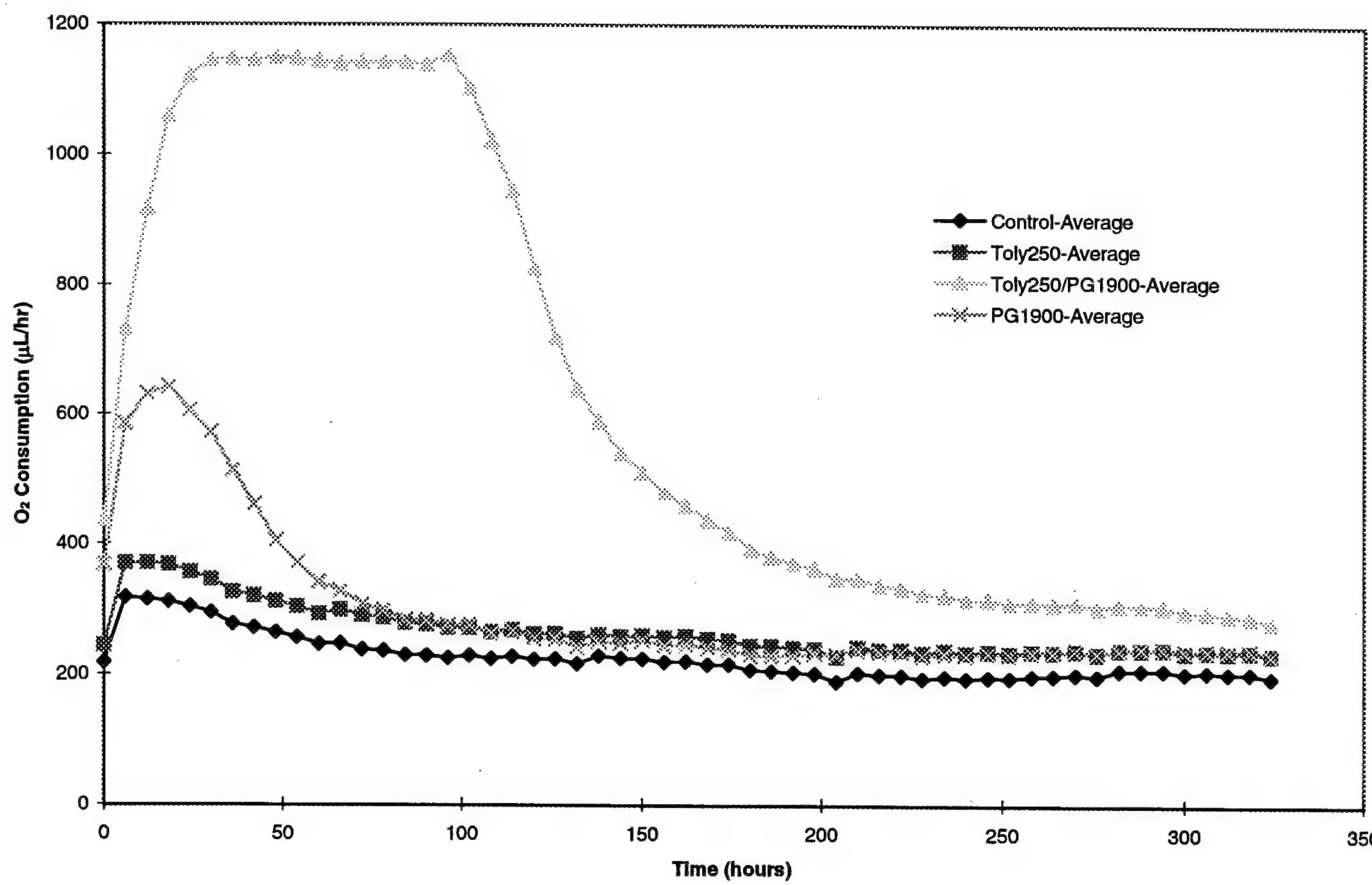


FIGURE C-7 Experiment 2 - Mean O_2 Consumption Rate for 25 mg/kg Tolyltriazole/1,900 mg/kg PG vs. 250 mg/kg Tolyltriazole/1,900 mg/kg PG vs. 1,900 mg/kg PG vs. the Control - All in High Clay Soil

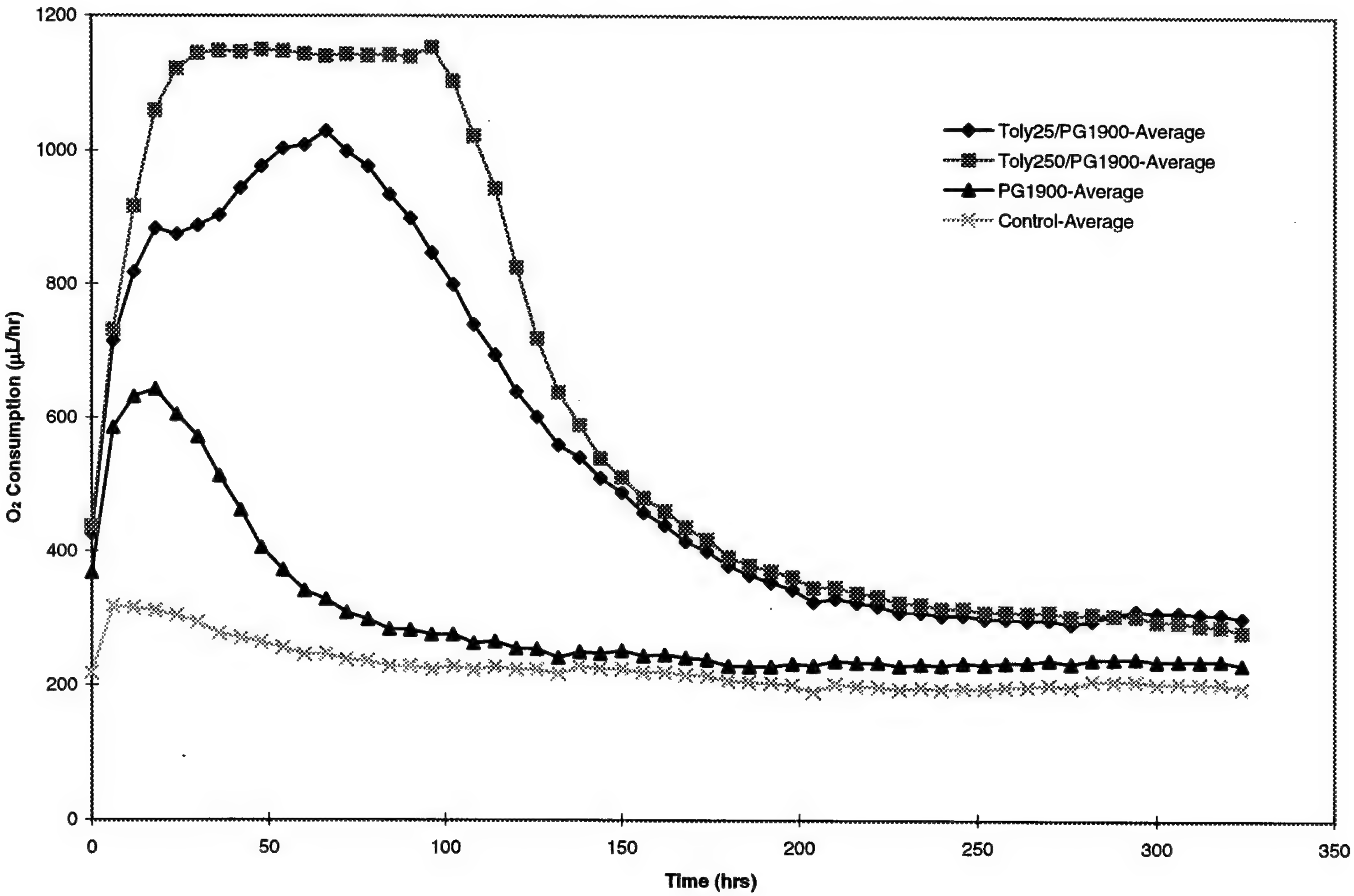


FIGURE C-8 Experiment 2 - Mean Cumulative O_2 Consumption for All Treatments in High Clay Soil

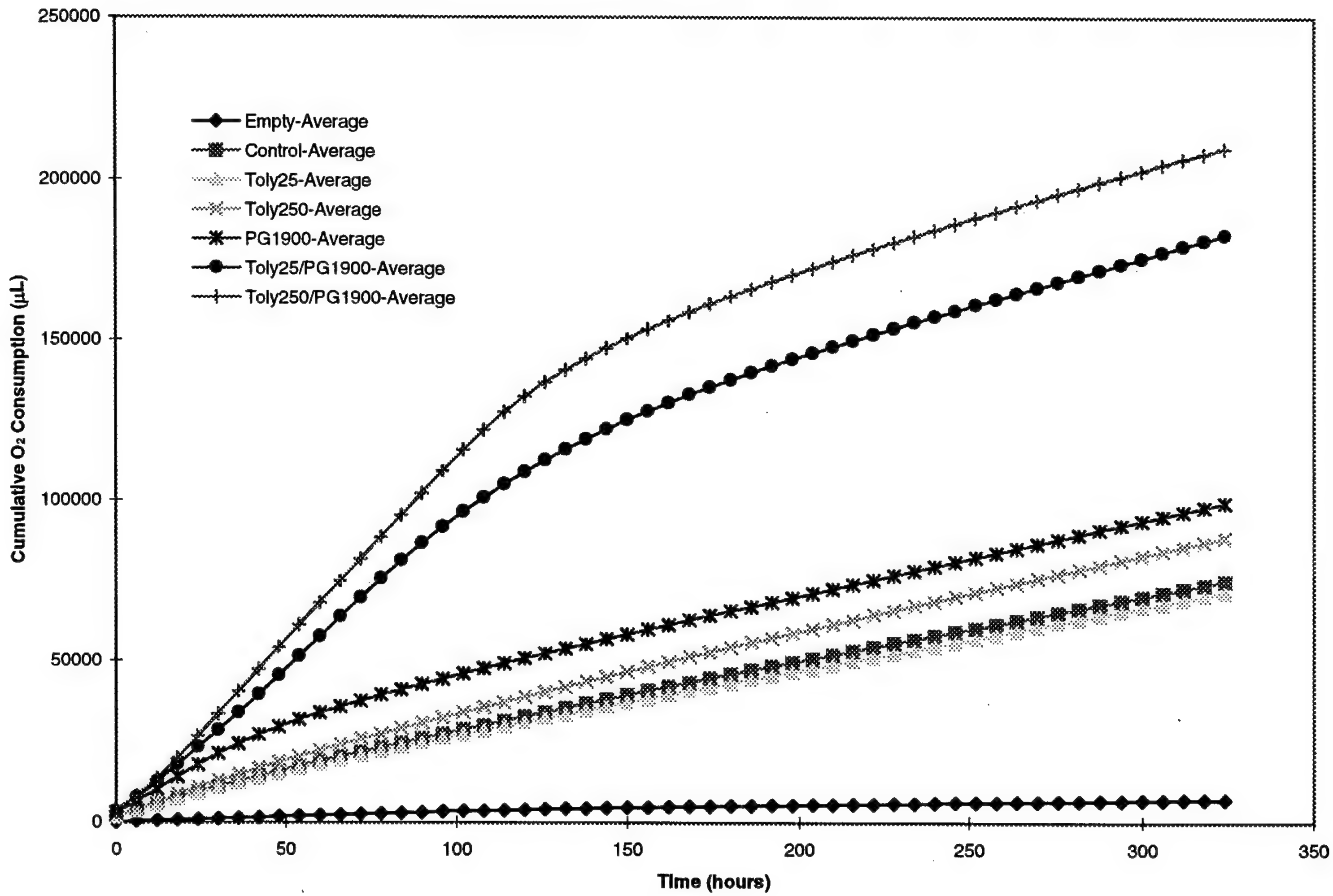


FIGURE C-9 Experiment 2 - Mean Cumulative O_2 Consumption for 25 mg/kg Tolyltriazole vs. 250 mg/kg Tolyltriazole vs. 1,900 mg/kg PG vs. the Control - All in High Clay Soil

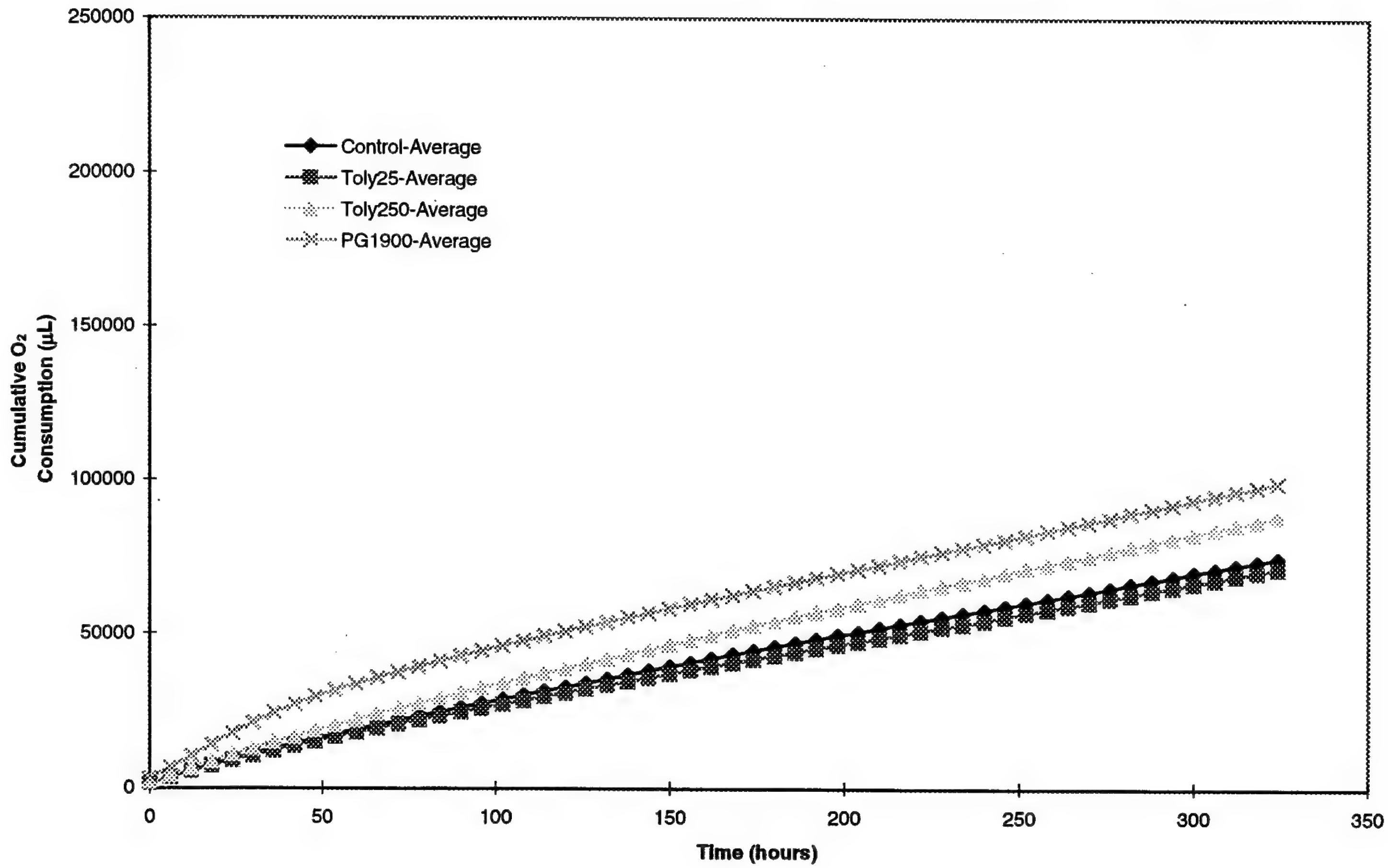


FIGURE C-10 Experiment 2 - Mean Cumulative O_2 Consumption for 25 mg/kg Tolyltriazole vs. 25 mg/kg Tolyltriazole/1,900 mg/kg PG vs. 1,900 mg/kg PG vs. the Control - All in High Clay Soil

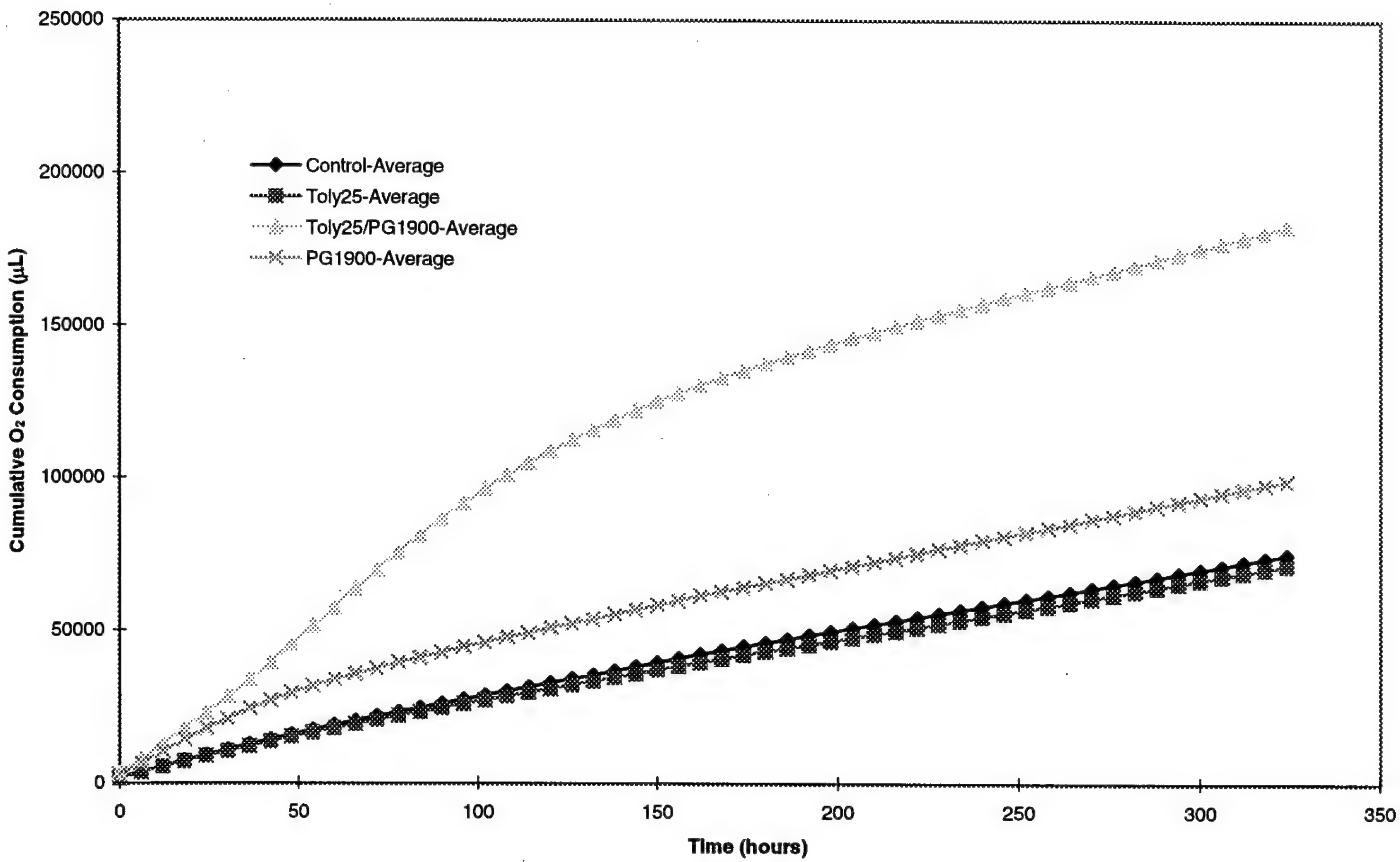


FIGURE C-11 Experiment 2 - Mean Cumulative O_2 Consumption for 250 mg/kg Tolyltriazole vs. 250 mg/kg Tolyltriazole/1,900 mg/kg PG vs. 1,900 mg/kg PG vs. the Control - All in High Clay Soil

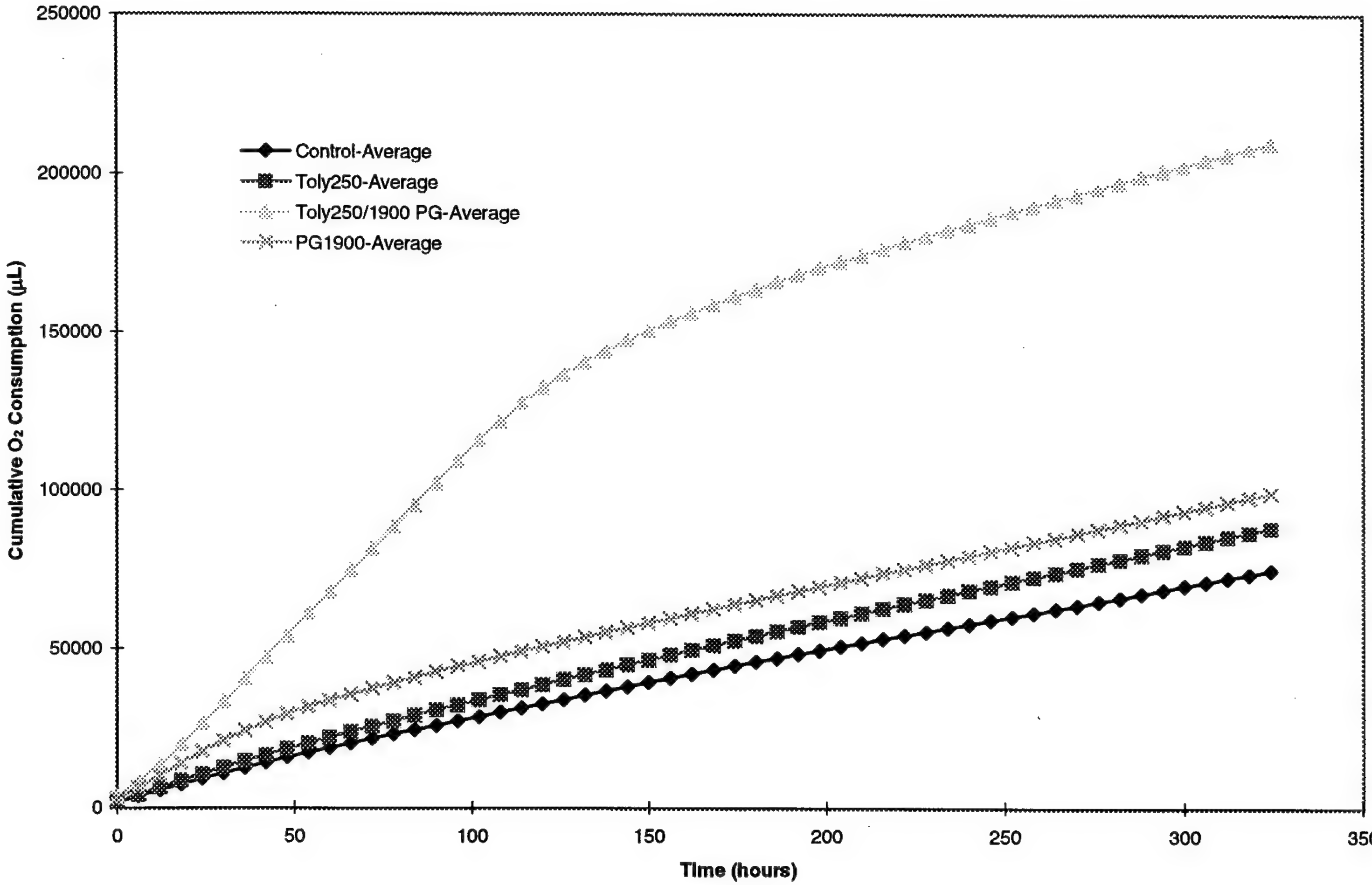
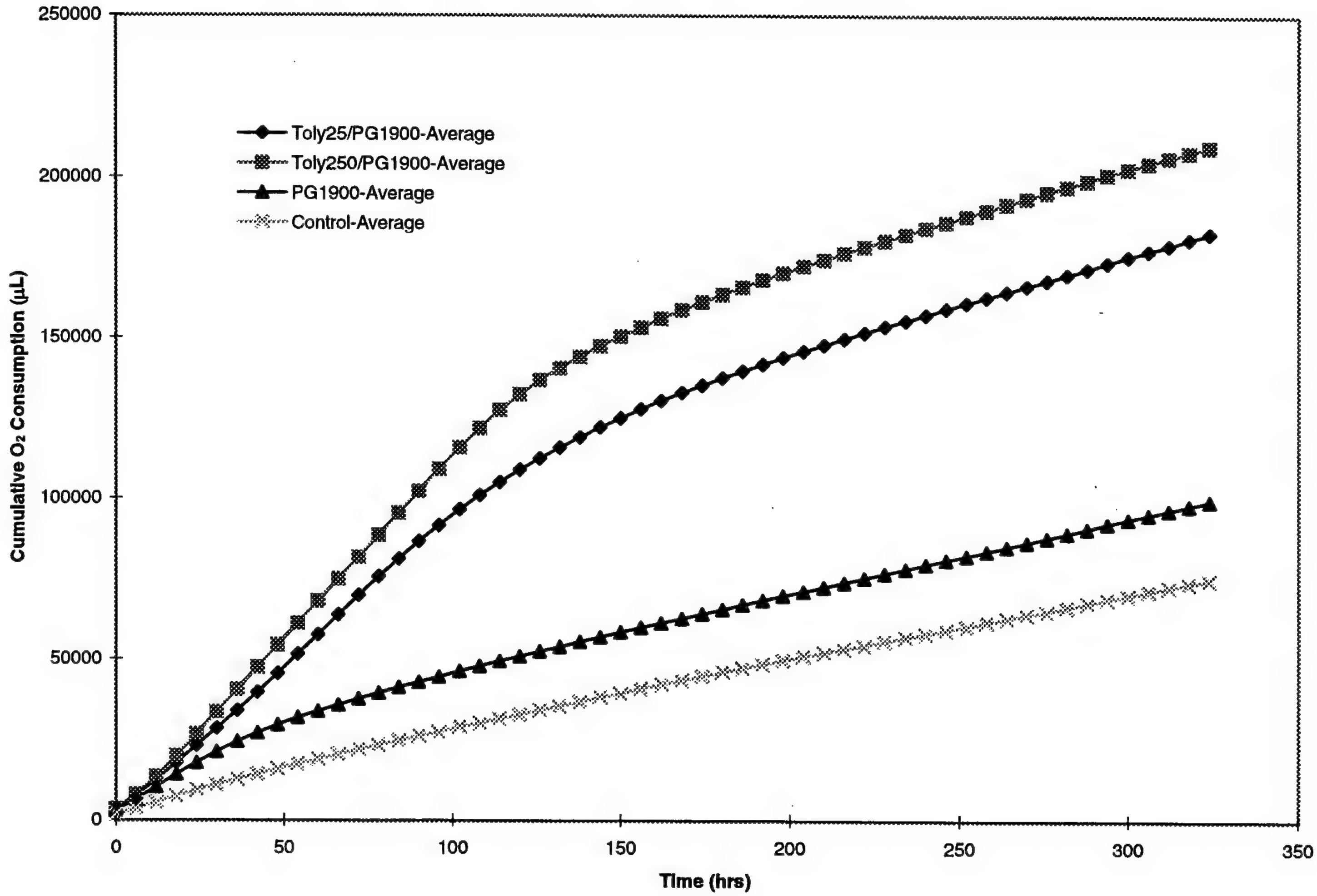


FIGURE C-12 Experiment 2 - Mean Cumulative O_2 Consumption for 25 mg/kg Tolyltriazole/1,900 mg/kg PG vs. 250 mg/kg Tolyltriazole/1,900 mg/kg PG vs. 1,900 mg/kg PG vs. the Control - All in High Clay Soil



APPENDIX D STATISTICAL DATA FOR DETERMINING WHETHER OR NOT MEASURABLE BIODEGRADATION OF TOLYLTRIAZOLE AND PROPYLENE GLYCOL OCCURRED

The following three tables and figures summarize the data used to determine whether or not biodegradation occurred in the microcosms contaminated with tolyltriazole and propylene glycol alone. This determination was made by comparing the oxygen consumption of the contaminated soil against the uncontaminated soil. The two sample t test and 95% confidence interval was used since both populations were assumed to be normal and the two population variances were assumed to be equal. The null hypothesis was that there was no effect on oxygen consumption due to contaminant addition.

The mean and standard deviation values on the tables were determined by taking the mean and standard deviation of the three microcosms for each treatment. The pooled estimator, which is an estimate of the common population variance was determined by using the following equation (4:358):

$$S_p2 = \frac{(n_1-1)^*S_1^2+(n_2-1)^*S_2^2}{n_1+n_2-2}$$

where n_1 and n_2 are the sample sizes of the two different treatments, and S_1 and S_2 are the standard deviations of the respective treatments.

The standard error was determined by the following equation (4:358):

$$\text{Std Error} = S_p^*(1/n_1+1/n_2)^{1/2}$$

The calculated t statistic was then determined by dividing the difference of the means by the standard error. The t-critical was determined for a two-tailed test since both degradation and inhibition were alternate hypotheses. The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t statistic to t-critical.

The upper and lower 95% confidence intervals were determined by using the following equation (4:361). This data is shown with the difference of the means in the Figures D-1, D-2, and D-3.

$$X_{\text{Toly or PG}}-X_{\text{Control}} \pm t_{\alpha/2,n1+n2-2}{}^*S_p{}^*(1/n_1+1/n_2)^{1/2}$$

List of Tables

List of Figures

TABLE D-1 Data for Determining Biodegradation of 25 mg/kg Tolyltriazole

Time (hours)	Mean O_2 Controls	STD DEV Controls	Mean O_2 Toly25	STD DEV Toly25	Pooled Estimator	Std Error	X_{Toly25} - $X_{Control}$	Calc T Value (T_{crit}=2.776303)	Upper 95% CI Pooled t	Lower 95% CI Pooled t	Biodegradation /Inhibition/No Effect
0	1717	112	1632	393	83485	236	-85	-0.361	570	-740	No Effect
6	3624	298	3550	793	358541	489	-73	-0.150	1284	-1431	No Effect
12	5515	511	5404	1176	822677	741	-111	-0.150	1945	-2167	No Effect
18	7384	716	7189	1542	1445460	982	-195	-0.199	2530	-2920	No Effect
24	9207	904	8888	1905	2223086	1217	-320	-0.263	3060	-3699	No Effect
30	10972	1075	10550	2229	3061130	1429	-421	-0.295	3544	-4387	No Effect
36	12631	1222	12108	2537	3963669	1626	-523	-0.322	3989	-5036	No Effect
42	14256	1343	13626	2830	4905762	1808	-630	-0.348	4390	-5650	No Effect
48	15840	1452	15117	3103	5870033	1978	-723	-0.365	4769	-6214	No Effect
54	17376	1533	16565	3364	6834240	2135	-811	-0.380	5115	-6736	No Effect
60	18850	1592	17955	3622	7824718	2284	-895	-0.392	5445	-7235	No Effect
66	20330	1629	19344	3875	8834637	2427	-986	-0.406	5751	-7723	No Effect
72	21758	1659	20686	4117	9851227	2563	-1072	-0.418	6042	-8186	No Effect
78	23174	1676	22013	4354	10882325	2693	-1162	-0.431	6315	-8639	No Effect
84	24548	1680	23295	4584	11919162	2819	-1254	-0.445	6572	-9079	No Effect
90	25921	1669	24587	4828	13047843	2949	-1334	-0.452	6853	-9522	No Effect
96	27270	1653	25862	5067	14201616	3077	-1408	-0.458	7134	-9950	No Effect
102	28642	1606	27140	5303	15351044	3199	-1502	-0.469	7379	-10382	No Effect
108	29982	1564	28383	5534	16535646	3320	-1599	-0.482	7618	-10816	No Effect
114	31349	1520	29651	5765	17775373	3442	-1698	-0.493	7858	-11254	No Effect
120	32688	1480	30890	5991	19038839	3563	-1798	-0.505	8092	-11688	No Effect
126	34033	1443	32131	6209	20315862	3680	-1902	-0.517	8314	-12118	No Effect
132	35332	1411	33326	6418	21592320	3794	-2006	-0.529	8526	-12539	No Effect
138	36702	1454	34559	6625	23003192	3916	-2143	-0.547	8728	-13014	No Effect
144	38052	1506	35782	6829	24455428	4038	-2270	-0.562	8939	-13479	No Effect
150	39397	1553	37019	7041	25991127	4163	-2378	-0.571	9177	-13933	No Effect
156	40713	1597	38232	7249	27547179	4285	-2481	-0.579	9415	-14377	No Effect
162	42031	1638	39452	7452	29111128	4405	-2579	-0.585	9651	-14808	No Effect
168	43326	1685	40658	7629	30523268	4511	-2668	-0.592	9854	-15191	No Effect
174	44617	1747	41853	7798	31930427	4614	-2764	-0.599	10043	-15572	No Effect

TABLE D-1 Data for Determining Biodegradation of 25 mg/kg Tolyltriazole

Time (hours)	Mean O_2 Controls	STD DEV Controls	Mean O_2 Toly25	STD DEV Toly25	Pooled Estimator	Std Error	$X_{Toly25} - X_{Control}$	Calc T Value (T_{crit}=2.776303)	Upper 95% CI Pooled t	Lower 95% CI Pooled t	Biodegradation /Inhibition/No Effect
180	45859	1803	43009	7957	33283151	4710	-2850	-0.605	10226	-15927	No Effect
186	47092	1858	44159	8097	34506478	4796	-2933	-0.612	10381	-16248	No Effect
192	48315	1915	45309	8233	35727988	4880	-3006	-0.616	10542	-16554	No Effect
198	49523	1969	46445	8360	36879371	4958	-3079	-0.621	10686	-16843	No Effect
204	50662	2025	47525	8524	38383664	5059	-3137	-0.620	10906	-17180	No Effect
210	51879	2081	48672	8620	39322047	5120	-3207	-0.626	11006	-17420	No Effect
216	53077	2135	49808	8725	40342880	5186	-3269	-0.630	11128	-17665	No Effect
222	54268	2187	50938	8829	41366789	5251	-3330	-0.634	11248	-17908	No Effect
228	55433	2232	52056	8930	42368583	5315	-3376	-0.635	11377	-18130	No Effect
234	56612	2285	53218	8970	42841128	5344	-3394	-0.635	11442	-18229	No Effect
240	57777	2342	54390	8983	43087182	5360	-3387	-0.632	11491	-18265	No Effect
246	58951	2400	55582	8986	43251440	5370	-3369	-0.627	11538	-18275	No Effect
252	60122	2459	56773	8989	43421589	5380	-3349	-0.622	11587	-18285	No Effect
258	61309	2526	57968	8997	43662007	5395	-3341	-0.619	11636	-18318	No Effect
264	62499	2600	59170	9006	43936020	5412	-3329	-0.615	11694	-18353	No Effect
270	63705	2675	60381	9018	44241820	5431	-3324	-0.612	11752	-18400	No Effect
276	64891	2759	61571	9032	44593070	5452	-3320	-0.609	11816	-18456	No Effect
282	66134	2827	62792	9041	44869643	5469	-3341	-0.611	11842	-18524	No Effect
288	67374	2899	64019	9052	45174603	5488	-3355	-0.611	11880	-18589	No Effect
294	68617	2978	65246	9068	45550252	5511	-3372	-0.612	11926	-18669	No Effect
300	69835	3056	66451	9087	45956668	5535	-3384	-0.611	11982	-18749	No Effect
306	71059	3142	67657	9111	46438538	5564	-3403	-0.612	12043	-18849	No Effect
312	72277	3226	68860	9132	46899187	5592	-3416	-0.611	12106	-18938	No Effect
318	73491	3314	70057	9152	47372589	5620	-3435	-0.611	12166	-19035	No Effect
324	74668	3427	71234	9173	47942491	5653	-3434	-0.607	12260	-19128	No Effect

FIGURE D-1 Difference Between the Means and 95% CI for 25 mg/kg Tolyltriazole

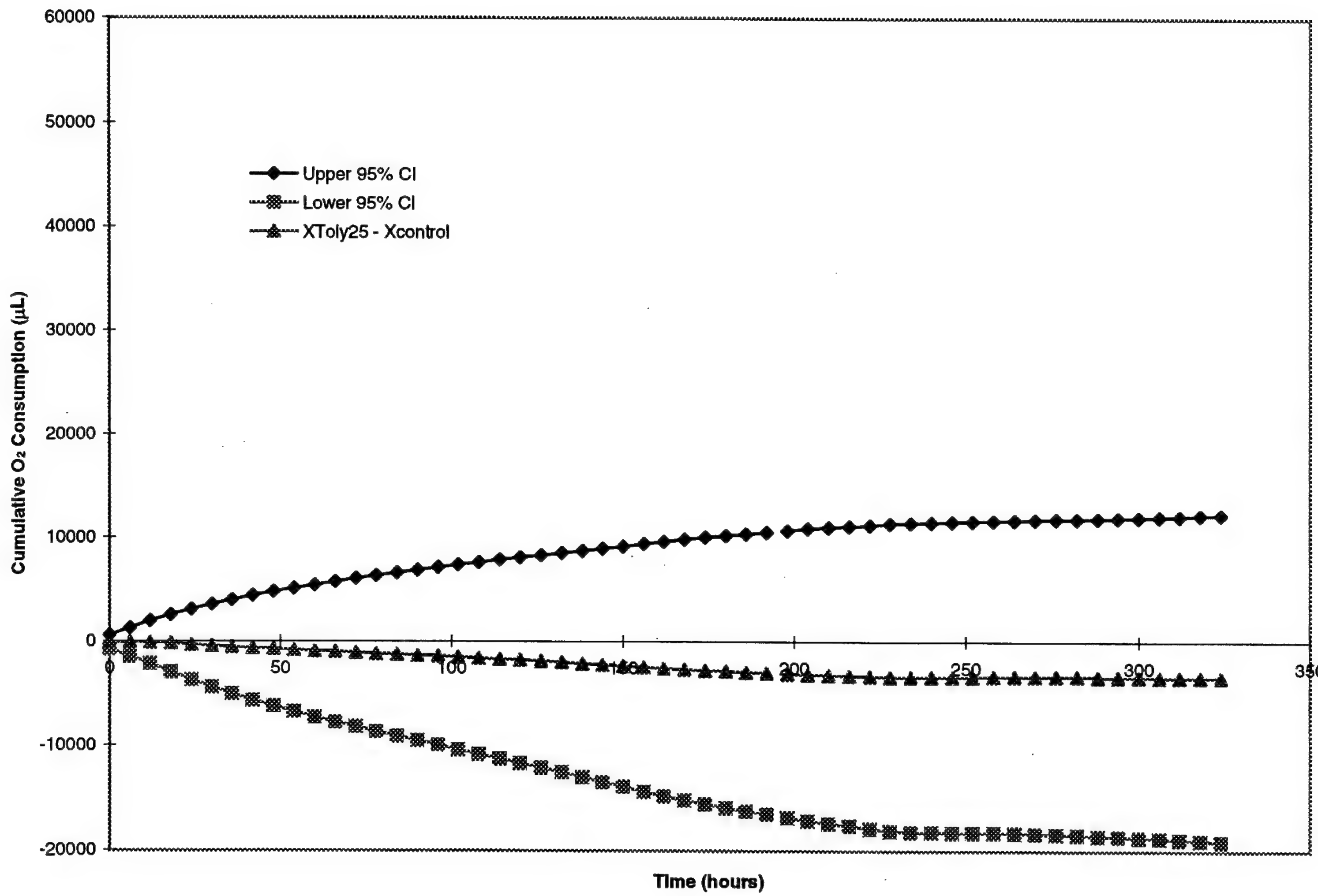


TABLE D-2 Data for Determining Biodegradation of 250 mg/kg Tolyltriazole

Time (hours)	Mean O_2 Controls	STD DEV Controls	Mean O_2 Toly250	STD DEV Toly250	Pooled Estimator	Std Error	$X_{Toly250} - X_{Control}$	Calc T Value (T_{crit}=2.776303)	Upper 95% CI Pooled t	Lowe. `5% CI Pooled t	Biodegradation/Inhibition/No Effect
0	1717	112	1924	348	66810	211	208	0.984	793	-378	No Effect
6	3624	298	4148	629	242252	402	524	1.305	1640	-591	No Effect
12	5515	511	6374	895	531547	595	859	1.443	2511	-794	No Effect
18	7384	716	8587	1146	912287	780	1203	1.543	3368	-961	No Effect
24	9207	904	10726	1372	1349238	948	1519	1.602	4152	-1114	No Effect
30	10972	1075	12799	1575	1818200	1101	1827	1.660	4883	-1229	No Effect
36	12631	1222	14755	1761	2296702	1237	2124	1.716	5559	-1311	No Effect
42	14256	1343	16680	1934	2772582	1360	2424	1.783	6198	-1351	No Effect
48	15840	1452	18552	2091	3240878	1470	2712	1.845	6793	-1368	No Effect
54	17376	1533	20376	2245	3695413	1570	3000	1.911	7357	-1357	No Effect
60	18850	1592	22133	2394	4133412	1660	3283	1.978	7892	-1325	No Effect
66	20330	1629	23928	2510	4476785	1728	3598	2.083	8394	-1198	No Effect
72	21758	1659	25670	2613	4789763	1787	3912	2.189	8873	-1048	No Effect
78	23174	1676	27392	2711	5078167	1840	4218	2.292	9326	-890	No Effect
84	24548	1680	29062	2807	5349740	1889	4514	2.390	9756	-729	No Effect
90	25921	1669	30721	2897	5590816	1931	4799	2.486	10159	-560	No Effect
96	27270	1653	32349	2977	5797322	1966	5079	2.584	10537	-378	No Effect
102	28642	1606	33977	3030	5881734	1980	5335	2.694	10832	-162	No Effect
108	29982	1564	35566	3073	5945237	1991	5583	2.804	11110	57	Biodegradation
114	31349	1520	37174	3110	5992693	1999	5825	2.914	11374	277	Biodegradation
120	32688	1480	38747	3140	6025196	2004	6059	3.023	11623	495	Biodegradation
126	34033	1443	40322	3167	6055851	2009	6289	3.130	11867	711	Biodegradation
132	35332	1411	41854	3198	6108444	2018	6521	3.232	12123	919	Biodegradation
138	36702	1454	43421	3223	6251402	2041	6719	3.291	12386	1052	Biodegradation
144	38052	1506	44972	3241	6386002	2063	6920	3.354	12648	1193	Biodegradation
150	39397	1553	46534	3259	6514725	2084	7138	3.425	12923	1352	Biodegradation
156	40713	1597	48080	3272	6628091	2102	7368	3.505	13203	1532	Biodegradation
162	42031	1638	49633	3276	6709176	2115	7602	3.594	13473	1731	Biodegradation
168	43326	1685	51157	3278	6792645	2128	7831	3.680	13738	1924	Biodegradation
174	44617	1747	52672	3273	6882112	2142	8055	3.760	14001	2108	Biodegradation

TABLE D-2 Data for Determining Biodegradation of 250 mg/kg Tolyltriazole

Time (hours)	Mean O_2 Controls	STD DEV Controls	Mean O_2 Toly250	STD DEV Toly250	Pooled Estimator	Std Error	$X_{Toly250}$ - $X_{Control}$	Calc T Value (T_{crit}=2.776303)	Upper 95% CI Pooled t	Lower 95% CI Pooled t	Biodegradation/ Inhibition/No Effect
180	45859	1803	54135	3269	6966902	2155	8276	3.840	14259	2293	Biodegradation
186	47092	1858	55599	3275	7086700	2174	8507	3.914	14541	2473	Biodegradation
192	48315	1915	57050	3283	7222872	2194	8735	3.980	14826	2643	Biodegradation
198	49523	1969	58487	3295	7368424	2216	8964	4.044	15116	2811	Biodegradation
204	50662	2025	59854	3326	7582809	2248	9192	4.088	15433	2950	Biodegradation
210	51879	2081	61306	3336	7731626	2270	9427	4.152	15729	3124	Biodegradation
216	53077	2135	62737	3357	7913764	2297	9660	4.206	16037	3284	Biodegradation
222	54268	2187	64167	3385	8120236	2327	9899	4.254	16358	3440	Biodegradation
228	55433	2232	65572	3419	8336066	2357	10140	4.301	16684	3596	Biodegradation
234	56612	2285	66998	3466	8618956	2397	10385	4.333	17040	3731	Biodegradation
240	57777	2342	68404	3514	8917288	2438	10626	4.358	17395	3858	Biodegradation
246	58951	2400	69820	3568	9247704	2483	10869	4.377	17762	3976	Biodegradation
252	60122	2459	71217	3622	9584576	2528	11095	4.389	18112	4078	Biodegradation
258	61309	2526	72630	3681	9964105	2577	11321	4.392	18475	4166	Biodegradation
264	62499	2600	74040	3736	10360584	2628	11541	4.391	18836	4245	Biodegradation
270	63705	2675	75461	3793	10770435	2680	11756	4.387	19195	4317	Biodegradation
276	64891	2759	76853	3853	11229881	2736	11962	4.372	19557	4366	Biodegradation
282	66134	2827	78286	3922	11687054	2791	12153	4.354	19901	4404	Biodegradation
288	67374	2899	79719	3999	12195065	2851	12345	4.330	20260	4430	Biodegradation
294	68617	2978	81158	4073	12729262	2913	12541	4.305	20628	4454	Biodegradation
300	69835	3056	82564	4148	13270268	2974	12729	4.280	20986	4472	Biodegradation
306	71059	3142	83978	4227	13870049	3041	12918	4.248	21359	4477	Biodegradation
312	72277	3226	85382	4312	14499968	3109	13105	4.215	21736	4474	Biodegradation
318	73491	3314	86795	4390	15125636	3175	13304	4.189	22119	4488	Biodegradation
324	74668	3427	88185	4481	15914351	3257	13517	4.150	22559	4475	Biodegradation

FIGURE D-2 Difference Between the Means and 95% CI for 250 mg/kg Tolyltriazole

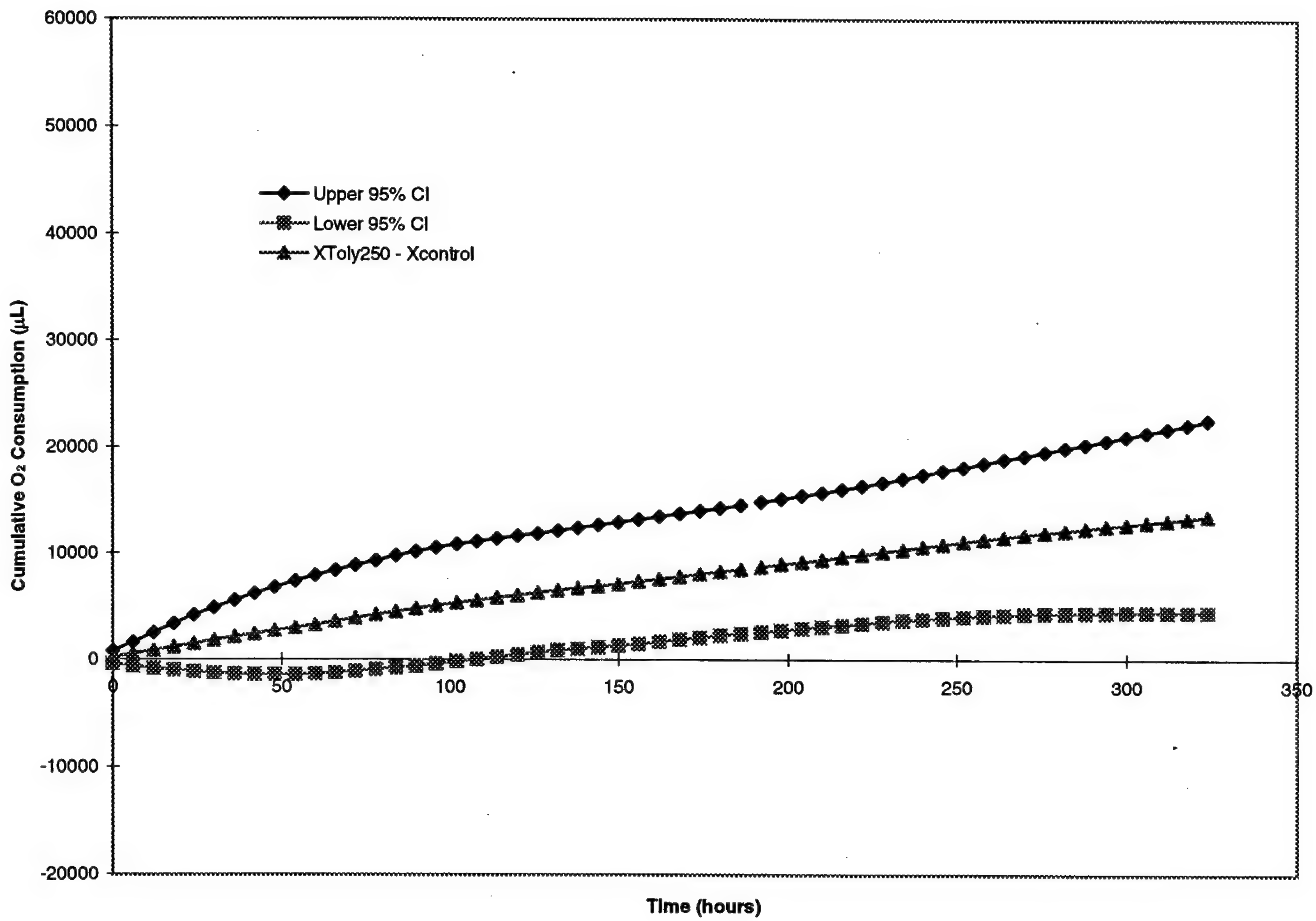


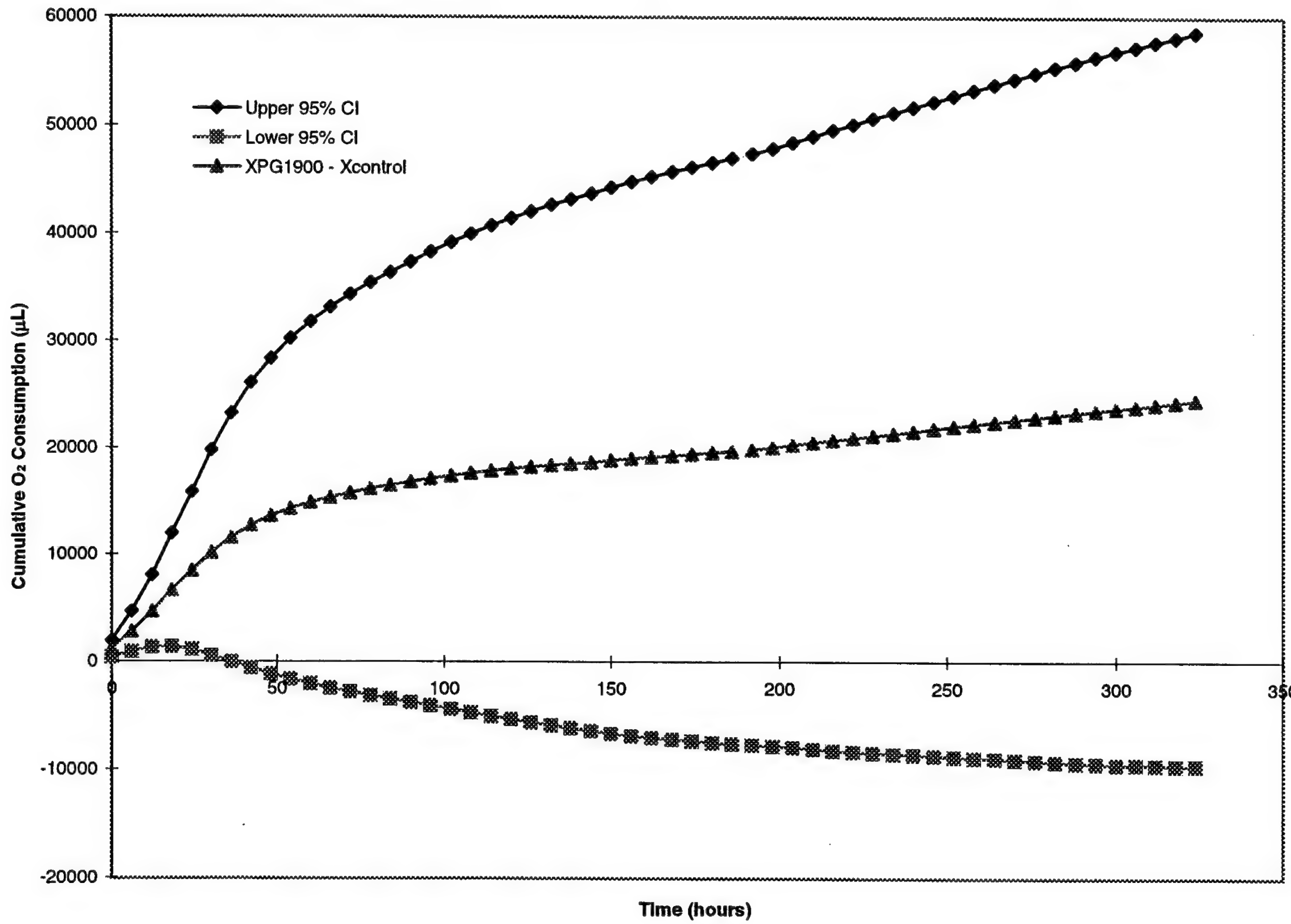
TABLE D-3 Data for Determinng Biodegradation of 1,900 mg/kg PG

Time (hours)	Mean O_2 Controls	STD DEV Controls	Mean O_2 PG1900	STD DEV PG1900	Pooled Estimator	Std Error	X_{PG1900} - $X_{Control}$	Calc T Value (T_{crit}=2.776)	Upper 95% CI Pooled t	Lower 95% CI Pooled t	Biodegradation/ Inhibition/No Effect
0	1717	112	2930	489	125632	289	1213	4.192	2017	410	Biodegradation
6	3624	298	6444	1131	684275	675	2820	4.175	4695	945	Biodegradation
12	5515	511	10235	2034	2199973	1211	4720	3.897	8082	1358	Biodegradation
18	7384	716	14092	3224	5454616	1907	6708	3.518	12002	1415	Biodegradation
24	9207	904	17724	4505	10554500	2653	8517	3.211	15880	1153	Biodegradation
30	10972	1075	21155	5875	17837592	3448	10184	2.953	19757	611	Biodegradation
36	12631	1222	24235	7134	26194348	4179	11604	2.777	23204	3	Biodegradation
42	14256	1343	27003	8198	34503449	4796	12747	2.658	26061	-567	No Effect
48	15840	1452	29439	9061	42101302	5298	13599	2.567	28306	-1108	No Effect
54	17376	1533	31671	9785	49044877	5718	14295	2.500	30169	-1578	No Effect
60	18850	1592	33717	10403	55382845	6076	14867	2.447	31735	-2001	No Effect
66	20330	1629	35686	10965	61441612	6400	15356	2.399	33123	-2411	No Effect
72	21758	1659	37535	11455	66984239	6683	15777	2.361	34328	-2773	No Effect
78	23174	1676	39323	11886	72047006	6930	16149	2.330	35388	-3090	No Effect
84	24548	1680	41025	12293	76972328	7163	16477	2.300	36363	-3409	No Effect
90	25921	1669	42721	12695	81973941	7393	16799	2.272	37321	-3723	No Effect
96	27270	1653	44376	13085	86967777	7614	17106	2.247	38244	-4031	No Effect
102	28642	1606	46029	13479	92129977	7837	17388	2.219	39143	-4368	No Effect
108	29982	1564	47608	13841	97010959	8042	17626	2.192	39951	-4699	No Effect
114	31349	1520	49202	14191	101848718	8240	17853	2.167	40727	-5022	No Effect
120	32688	1480	50734	14496	106159381	8413	18046	2.145	41399	-5308	No Effect
126	34033	1443	52258	14789	110392667	8579	18225	2.124	42039	-5590	No Effect
132	35332	1411	53708	15072	114584165	8740	18376	2.102	42638	-5887	No Effect
138	36702	1454	55209	15322	118442156	8886	18507	2.083	43175	-6161	No Effect
144	38052	1506	56696	15561	122212166	9026	18645	2.066	43702	-6412	No Effect
150	39397	1553	58207	15799	126016604	9166	18810	2.052	44254	-6634	No Effect
156	40713	1597	59673	16028	129728708	9300	18960	2.039	44776	-6856	No Effect
162	42031	1638	61148	16235	133133124	9421	19117	2.029	45270	-7036	No Effect
168	43326	1685	62596	16419	136218360	9530	19270	2.022	45724	-7184	No Effect
174	44617	1747	64028	16595	139214110	9634	19411	2.015	46154	-7333	No Effect

TABLE D-3 Data for Determinng Biodegradation of 1,900 mg/kg PG

Time (hours)	Mean O_2 Controls	STD DEV Controls	Mean O_2 PG1900	STD DEV PG1900	Pooled Estimator	Std Error	$X_{PG1900} - X_{Control}$	Calc T Value (T_{crit}=2.776)	Upper 95% CI Pooled t	Lower 95% CI Pooled t	Biodegradation/ Inhibition/No Effect
180	45859	1803	65405	16758	142047852	9731	19546	2.009	46560	-7468	No Effect
186	47092	1858	66779	16917	144823644	9826	19687	2.004	46964	-7590	No Effect
192	48315	1915	68151	17070	147532316	9917	19836	2.000	47367	-7695	No Effect
198	49523	1969	69547	17257	150835836	10028	20023	1.997	47861	-7814	No Effect
204	50662	2025	70929	17460	154473087	10148	20266	1.997	48437	-7905	No Effect
210	51879	2081	72353	17680	158460798	10278	20474	1.992	49006	-8059	No Effect
216	53077	2135	73760	17892	162335525	10403	20683	1.988	49562	-8195	No Effect
222	54268	2187	75170	18097	166142956	10524	20902	1.986	50117	-8314	No Effect
228	55433	2232	76547	18294	169820691	10640	21115	1.984	50652	-8422	No Effect
234	56612	2285	77940	18480	173370830	10751	21328	1.984	51172	-8516	No Effect
240	57777	2342	79324	18670	177029793	10864	21547	1.983	51704	-8611	No Effect
246	58951	2400	80729	18864	180799184	10979	21778	1.984	52255	-8699	No Effect
252	60122	2459	82114	19052	184505784	11091	21992	1.983	52780	-8795	No Effect
258	61309	2526	83518	19233	188138298	11199	22209	1.983	53298	-8881	No Effect
264	62499	2600	84924	19408	191709004	11305	22425	1.984	53808	-8958	No Effect
270	63705	2675	86353	19594	195541815	11418	22649	1.984	54344	-9047	No Effect
276	64891	2759	87752	19776	199355110	11528	22861	1.983	54864	-9141	No Effect
282	66134	2827	89187	19954	203083142	11636	23053	1.981	55354	-9247	No Effect
288	67374	2899	90624	20130	206807683	11742	23250	1.980	55845	-9346	No Effect
294	68617	2978	92069	20300	210485181	11846	23452	1.980	56336	-9432	No Effect
300	69835	3056	93488	20460	213965509	11943	23653	1.980	56808	-9501	No Effect
306	71059	3142	94909	20597	217046588	12029	23850	1.983	57243	-9543	No Effect
312	72277	3226	96329	20738	220243381	12117	24052	1.985	57690	-9585	No Effect
318	73491	3314	97753	20873	223339516	12202	24262	1.988	58135	-9612	No Effect
324	74668	3427	99139	21006	226492373	12288	24471	1.991	58583	-9640	No Effect

FIGURE D-3 Difference Between the Means and 95% CI for 1,900 mg/kg PG



APPENDIX E STATISTICAL DATA FOR DETERMINING WHETHER OR NOT MEASURABLE BIODEGRADATION OCCURRED IN THE TREATMENTS OF COMBINED TOLYLTRIAZOLE AND PROPYLENE GLYCOL

The following two tables and figures summarize the data used to determine whether or not biodegradation occurred in the microcosms contaminated with both tolyltriazole and PG. This determination was made by comparing the oxygen consumption of the soil contaminated with both PG and tolyltriazole against soil contaminated with only PG, soil contaminated with only tolyltriazole and the uncontaminated soil. The t test and 95% confidence interval for a linear combination was used since all populations were assumed to be normal and all the population variances were assumed to be equal. The null hypothesis was that there was no effect on oxygen consumption due to combining the two contaminants.

The mean and standard deviation values on the tables were determined by taking the mean and standard deviation of the three microcosms for each treatment. The pooled estimator, which is an estimate of the common population variance was determined by using the following equation (4:358):

$$S_p^2 = \frac{(n_1-1)*S_1^2+(n_2-1)*S_2^2+(n_3-1)*S_3^2+(n_4-1)*S_4^2}{n_1+n_2+n_3+n_4-2}$$

where n_1, n_2, n_3, and n_4 are the sample sizes of the different treatments, and S_1, S_2, S_3, and S_4 are the standard deviations of the respective treatments.

The standard error was determined by the following equation (4:359):

$$\text{Std Error} = S_p*(1/n_1+1/n_2+1/n_3+1/n_4)^{1/2}$$

The calculated t statistic was then determined by dividing the difference of the means by the standard error. T critical was determined for a two-tailed test since both degradation and inhibition were alternate hypotheses. The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t statistic to t critical.

The upper and lower 95% confidence intervals were determined by using the following equation (4:361). This data is shown with the difference of the means in Figures E-1 and E-2.

$$X_{PG/Toly}-X_{Toly}-X_{PG}+X_{Control} \pm t_{\alpha/2,n1+n2+n3+n4-2}*S_p*(1/n_1+1/n_2+1/n_3+1/n_4)^{1/2}$$

List of Tables

List of Figures

TABLE E-1 Data for Determining Biodegradation for the Combined Treatment of 25 mg/kg Tolyltriazole and 1,900 mg/kg PG

Time (hrs)	Mean Control	Std Dev Control	Mean PG1900	Std Dev PG1900	Mean Toly25	Std Dev Toly25	Mean Toly25/ PG1900	Std Dev Toly25/ PG1900	Pooled Estimator	Std Error	$X_{Toly25/PG1900} - X_{Toly25} - X_{PG1900} + X_{Control}$	Calc T Value (T_{crit}= 2.228)	Upper 95% CI	Lower 95% CI	Biodegradation/ Inhibition/No Effect
0	1717	112	2930	489	1632	393	3289	218	90603	348	445	1.279	1219	-330	No Effect
6	3624	298	6444	1131	3550	793	7579	371	426930	754	1208	1.602	2889	-473	No Effect
12	5515	511	10235	2034	5404	1176	12491	574	1222702	1277	2368	1.854	5212	-477	No Effect
18	7384	716	14092	3224	7189	1542	17796	803	2786383	1927	3899	2.023	8193	-396	No Effect
24	9207	904	17724	4505	8888	1905	23045	1057	5170996	2626	5641	2.148	11491	-209	No Effect
30	10972	1075	21155	5875	10550	2229	28372	1331	8482769	3363	7638	2.271	15131	145	Biodegradation
36	12631	1222	24235	7134	12108	2537	33793	1701	12343303	4057	10081	2.485	19120	1043	Biodegradation
42	14256	1343	27003	8198	13626	2830	39458	2090	16276048	4658	13085	2.809	23464	2705	Biodegradation
48	15840	1452	29439	9061	15117	3103	45318	2552	20068639	5173	16602	3.209	28127	5076	Biodegradation
54	17376	1533	31671	9785	16565	3364	51339	3070	23767304	5629	20478	3.638	33021	7936	Biodegradation
60	18850	1592	33717	10403	17955	3622	57387	3633	27416684	6046	24565	4.063	38036	11094	Biodegradation
66	20330	1629	35686	10965	19344	3875	63560	4272	31230043	6453	28861	4.473	43238	14484	Biodegradation
72	21758	1659	37535	11455	20686	4117	69554	4894	34974889	6829	33091	4.846	48305	17876	Biodegradation
78	23174	1676	39323	11886	22013	4354	75415	5465	38583898	7173	37253	5.194	53234	21273	Biodegradation
84	24548	1680	41025	12293	23295	4584	81021	5977	42137014	7496	41250	5.503	57950	24550	Biodegradation
90	25921	1669	42721	12695	24587	4828	86419	6324	45450432	7785	45032	5.785	62377	27688	Biodegradation
96	27270	1653	44376	13085	25862	5067	91503	6478	48315215	8026	48535	6.047	66418	30653	Biodegradation
102	28642	1606	46029	13479	27140	5303	96304	6387	50635875	8217	51776	6.301	70083	33469	Biodegradation
108	29982	1564	47608	13841	28383	5534	100746	6141	52471823	8364	54737	6.544	73372	36101	Biodegradation
114	31349	1520	49202	14191	29651	5765	104911	5755	54012190	8486	57407	6.765	76315	38500	Biodegradation
120	32688	1480	50734	14496	30890	5991	108746	5217	55084706	8570	59810	6.979	78904	40716	Biodegradation
126	34033	1443	52258	14789	32131	6209	112357	4636	56166057	8654	62002	7.165	81282	42721	Biodegradation
132	35332	1411	53708	15072	33326	6418	115714	4059	57368424	8746	64012	7.319	83498	44526	Biodegradation
138	36702	1454	55209	15322	34559	6625	118959	3583	58722641	8849	65893	7.447	85608	46179	Biodegradation
144	38052	1506	56696	15561	35782	6829	122018	3255	60331745	8969	67592	7.536	87575	47609	Biodegradation
150	39397	1553	58207	15799	37019	7041	124949	3043	62173034	9105	69120	7.592	89406	48835	Biodegradation
156	40713	1597	59673	16028	38232	7249	127700	2963	64155736	9249	70508	7.623	91115	49902	Biodegradation
162	42031	1638	61148	16235	39452	7452	130337	2952	66103688	9388	71767	7.644	92684	50850	Biodegradation
168	43326	1685	62596	16419	40658	7629	132828	3006	67935567	9517	72900	7.660	94105	51696	Biodegradation
174	44617	1747	64028	16595	41853	7798	135231	3087	69753516	9644	73968	7.670	95454	52481	Biodegradation
180	45859	1803	65405	16758	43009	7957	137504	3178	71501849	9764	74949	7.676	96703	53195	Biodegradation
186	47092	1858	66779	16917	44159	8097	139697	3275	73187013	9878	75851	7.678	97860	53842	Biodegradation
192	48315	1915	68151	17070	45309	8233	141821	3354	74820801	9988	76676	7.677	98929	54423	Biodegradation

TABLE E-1 Data for Determining Biodegradation for the Combined Treatment of 25 mg/kg Tolyltriazole and 1,900 mg/kg PG

Time (hrs)	Mean Control	Std Dev Control	Mean PG1900	Std Dev PG1900	Mean Toly25	Std Dev Toly25	Mean Toly25/ PG1900	Std Dev Toly25/ PG1900	Pooled Estimator	Std Error	$X_{Toly25/PG1900}-X_{Toly25}-X_{PG1900}+X_{Control}$	Calc T Value (T_{crit}= 2.228)	Upper 95% CI	Lower 95% CI	Biodegradation/ Inhibition/No Effect
198	49523	1969	69547	17257	46445	8360	143884	3425	76657447	10110	77415	7.657	99940	54891	Biodegradation
204	50662	2025	70929	17460	47525	8524	145833	3492	78761882	10248	78041	7.615	100873	55209	Biodegradation
210	51879	2081	72353	17680	48672	8620	147816	3574	80801009	10380	78670	7.579	101796	55545	Biodegradation
216	53077	2135	73760	17892	49808	8725	149760	3651	82825861	10509	79269	7.543	102682	55855	Biodegradation
222	54268	2187	75170	18097	50938	8829	151675	3724	84821067	10635	79834	7.507	103528	56141	Biodegradation
228	55433	2232	76547	18294	52056	8930	153533	3781	86738770	10754	80361	7.473	104322	56401	Biodegradation
234	56612	2285	77940	18480	53218	8970	155388	3825	88366958	10855	80841	7.448	105025	56657	Biodegradation
240	57777	2342	79324	18670	54390	8983	157211	3867	89941262	10951	81274	7.422	105673	56876	Biodegradation
246	58951	2400	80729	18864	55582	8986	159044	3927	91552497	11049	81684	7.393	106300	57068	Biodegradation
252	60122	2459	82114	19052	56773	8989	160845	3987	93139694	11144	82080	7.365	106908	57251	Biodegradation
258	61309	2526	83518	19233	57968	8997	162644	4053	94729752	11239	82467	7.338	107507	57427	Biodegradation
264	62499	2600	84924	19408	59170	9006	164430	4117	96296594	11331	82836	7.310	108081	57590	Biodegradation
270	63705	2675	86353	19594	60381	9018	166222	4184	97983041	11430	83192	7.278	108658	57726	Biodegradation
276	64891	2759	87752	19776	61571	9032	167977	4247	99663215	11528	83545	7.247	109228	57862	Biodegradation
282	66134	2827	89187	19954	62792	9041	169759	4302	101283943	11621	83913	7.221	109805	58022	Biodegradation
288	67374	2899	90624	20130	64019	9052	171595	4402	102988509	11718	84326	7.196	110435	58218	Biodegradation
294	68617	2978	92069	20300	65246	9068	173470	4540	104762186	11819	84772	7.173	111104	58440	Biodegradation
300	69835	3056	93488	20460	66451	9087	175321	4691	106503528	11917	85217	7.151	111767	58666	Biodegradation
306	71059	3142	94909	20597	67657	9111	177180	4860	108144258	12008	85673	7.135	112427	58919	Biodegradation
312	72277	3226	96329	20738	68860	9132	179023	5027	109830091	12101	86110	7.116	113072	59149	Biodegradation
318	73491	3314	97753	20873	70057	9152	180862	5199	111494529	12193	86544	7.098	113709	59379	Biodegradation
324	74668	3427	99139	21006	71234	9173	182668	5377	113207512	12286	86963	7.078	114336	59590	Biodegradation

FIGURE E-1 Difference Between the Means and 95% CI for the Linear Combination of 25 mg/kg Tolyltriazole and 1,900 mg/kg PG

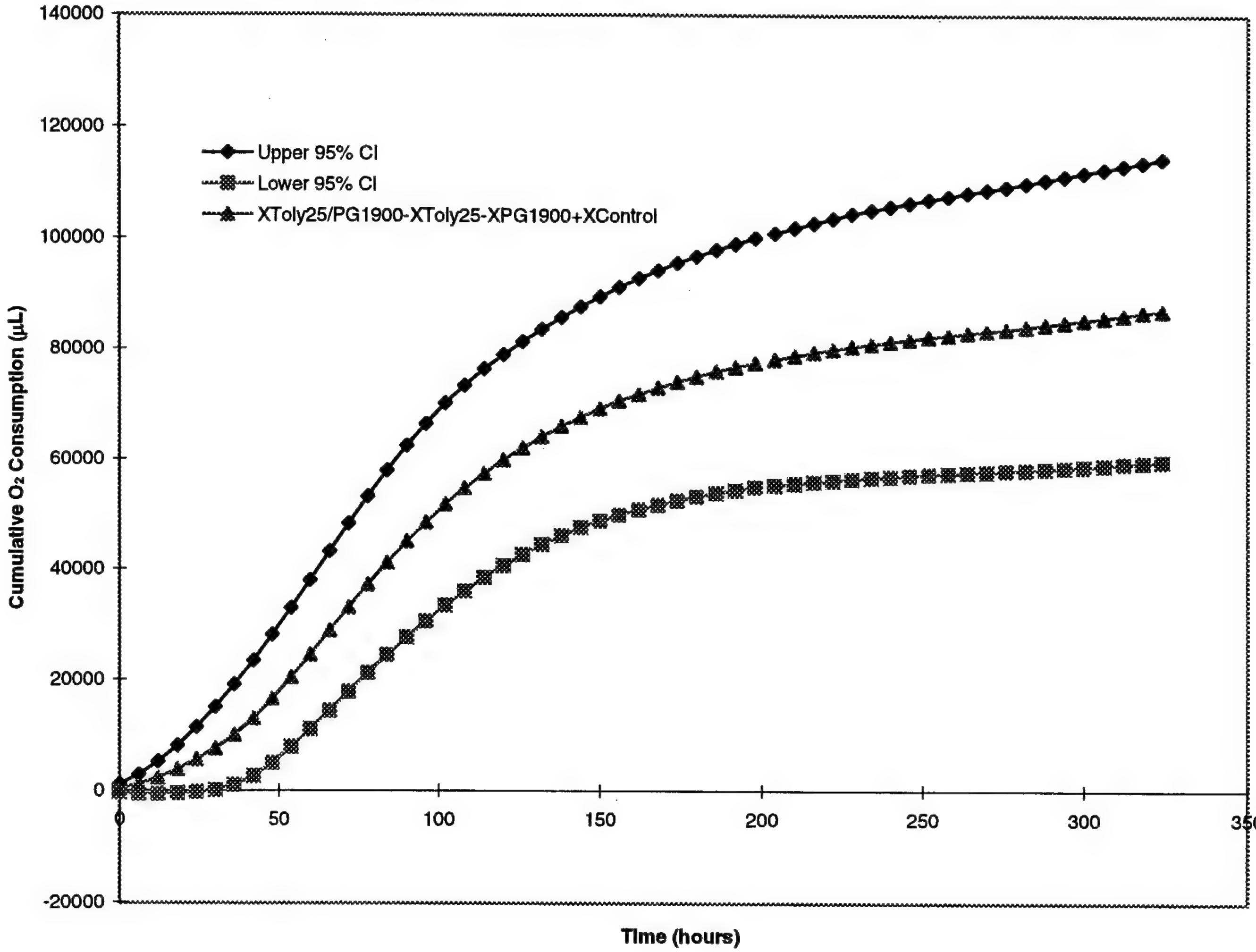


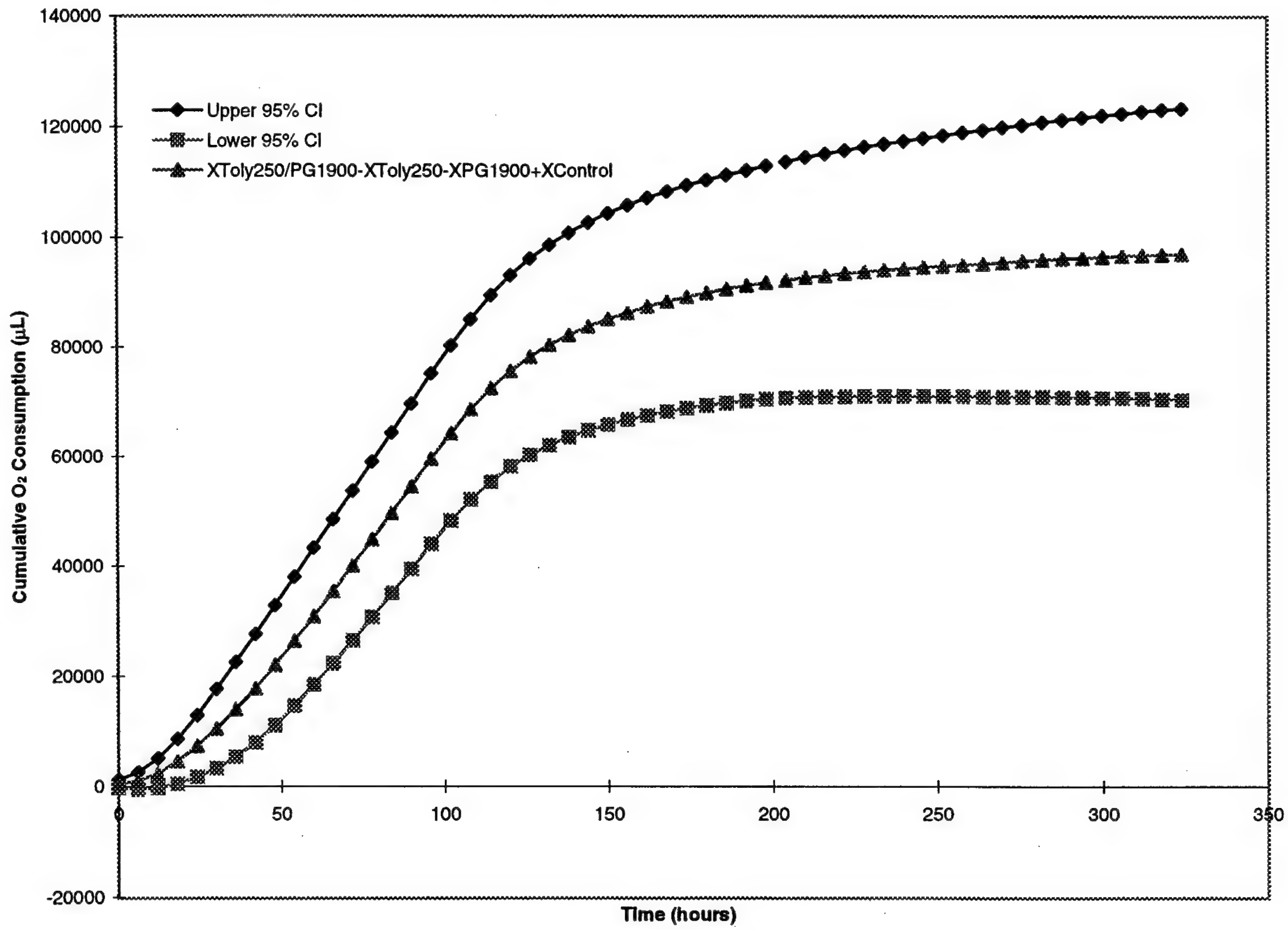
TABLE E-2 Data for Determining Biodegradation of the Combined Treatment of 250 mg/kg Tolyltriazole and 1,900 mg/kg PG

Time (hrs)	Mean Control	Std Dev Control	Mean PG1900	Std Dev PG1900	Mean Toly250	Std Dev Toly250	Mean Toly250/ PG1900	Std Dev Toly250/ PG1900	Pooled Estimator	Std Error	$X_{Toly250/PG1900}-X_{Toly250}-X_{PG1900}+X_{control}$	Calc T Value (T_{crit}= 2.228)	Upper 95% CI	Lower 95% CI	Biodegradation/ Inhibition/No Effect
0	1717	112	2930	489	1924	348	3604	111	76909	320	467	1.457	1180	-247	No Effect
6	3624	298	6444	1131	4148	629	7996	172	358792	692	1028	1.486	2569	-513	No Effect
12	5515	511	10235	2034	6374	895	13501	338	1063236	1191	2407	2.022	5060	-245	No Effect
18	7384	716	14092	3224	8587	1146	19860	522	2498742	1825	4565	2.501	8631	498	Biodegradation
24	9207	904	17724	4505	10726	1372	26591	655	4684052	2499	7348	2.940	12916	1780	Biodegradation
30	10972	1075	21155	5875	12799	1575	33464	668	7720289	3208	10481	3.267	17630	3333	Biodegradation
36	12631	1222	24235	7134	14755	1761	40354	588	11166932	3859	13995	3.627	22592	5398	Biodegradation
42	14256	1343	27003	8198	16680	1934	47232	503	14600046	4412	17806	4.036	27636	7976	Biodegradation
48	15840	1452	29439	9061	18552	2091	54131	416	17749583	4865	21980	4.518	32819	11141	Biodegradation
54	17376	1533	31671	9785	20376	2245	61020	324	20647210	5247	26349	5.022	38039	14659	Biodegradation
60	18850	1592	33717	10403	22133	2394	67884	257	23312915	5575	30883	5.539	43305	18461	Biodegradation
66	20330	1629	35686	10965	23928	2510	74728	159	25841495	5870	35444	6.038	48523	22366	Biodegradation
72	21758	1659	37535	11455	25670	2613	81590	84	28160591	6128	40143	6.551	53795	26491	Biodegradation
78	23174	1676	39323	11886	27392	2711	88439	72	30289544	6355	44897	7.065	59056	30738	Biodegradation
84	24548	1680	41025	12293	29062	2807	95292	109	32366684	6569	49753	7.574	64389	35116	Biodegradation
90	25921	1669	42721	12695	30721	2897	102131	194	34476133	6780	54611	8.055	69717	39506	Biodegradation
96	27270	1653	44376	13085	32349	2977	109052	271	36574477	6983	59596	8.534	75155	44037	Biodegradation
102	28642	1606	46029	13479	33977	3030	115673	469	38732713	7186	64308	8.949	80319	48297	Biodegradation
108	29982	1564	47608	13841	35566	3073	121803	1141	40953455	7389	68612	9.285	85076	52148	Biodegradation
114	31349	1520	49202	14191	37174	3110	127469	2051	43515545	7617	72442	9.510	89413	55471	Biodegradation
120	32688	1480	50734	14496	38747	3140	132423	2717	45912615	7824	75630	9.666	93062	58198	Biodegradation
126	34033	1443	52258	14789	40322	3167	136742	3196	48206255	8017	78195	9.753	96057	60333	Biodegradation
132	35332	1411	53708	15072	41854	3198	140575	3541	50386471	8196	80346	9.803	98608	62084	Biodegradation
138	36702	1454	55209	15322	43421	3223	144116	3808	52355088	8355	82188	9.837	100803	63573	Biodegradation
144	38052	1506	56696	15561	44972	3241	147354	3969	54136528	8496	83737	9.856	102666	64808	Biodegradation
150	39397	1553	58207	15799	46534	3259	150421	4114	55915212	8634	85076	9.853	104313	65838	Biodegradation
156	40713	1597	59673	16028	48080	3272	153302	4251	57646493	8767	86262	9.839	105795	66729	Biodegradation
162	42031	1638	61148	16235	49633	3276	156067	4388	59251727	8888	87318	9.824	107121	67514	Biodegradation
168	43326	1685	62596	16419	51157	3278	158690	4527	60735279	8999	88262	9.808	108312	68213	Biodegradation
174	44617	1747	64028	16595	52672	3273	161201	4683	62214107	9108	89119	9.785	109411	68827	Biodegradation
180	45859	1803	65405	16758	54135	3269	163551	4826	63613424	9210	89870	9.758	110389	69351	Biodegradation
186	47092	1858	66779	16917	55599	3275	165832	4967	65008352	9310	90546	9.726	111288	69803	Biodegradation
192	48315	1915	68151	17070	57050	3283	168062	5137	66445812	9412	91176	9.687	112147	70205	Biodegradation

TABLE E-2 Data for Determining Biodegradation of the Combined Treatment of 250 mg/kg Tolyltriazole and 1,900 mg/kg PG

Time (hrs)	Mean Control	Std Dev Control	Mean PG1900	Std Dev PG1900	Mean Toly250	Std Dev Toly250	Mean Toly250/ PG1900	Std Dev Toly250/ PG1900	Pooled Estimator	Std Error	$X_{Toly250/PG1900}-X_{Toly250}-X_{PG1900}+X_{control}$	Calc T Value (T_{crit}= 2.228)	Upper 95% CI	Lower 95% CI	Biodegradation/ Inhibition/No Effect
198	49523	1969	69547	17257	58487	3295	170239	5320	68167651	9534	91728	9.622	112969	70487	Biodegradation
204	50662	2025	70929	17460	59854	3326	172317	5500	70053155	9665	92197	9.540	113730	70665	Biodegradation
210	51879	2081	72353	17680	61306	3336	174403	5674	72049101	9801	92624	9.450	114461	70787	Biodegradation
216	53077	2135	73760	17892	62737	3357	176434	5838	74004708	9933	93014	9.364	115145	70882	Biodegradation
222	54268	2187	75170	18097	64167	3385	178433	5997	75941821	10063	93365	9.278	115784	70945	Biodegradation
228	55433	2232	76547	18294	65572	3419	180376	6146	77820577	10186	93689	9.198	116384	70994	Biodegradation
234	56612	2285	77940	18480	66998	3466	182308	6286	79653630	10306	93982	9.120	116943	71021	Biodegradation
240	57777	2342	79324	18670	68404	3514	184202	6407	81492840	10424	94251	9.042	117476	71027	Biodegradation
246	58951	2400	80729	18864	69820	3568	186097	6519	83366789	10543	94499	8.963	117989	71009	Biodegradation
252	60122	2459	82114	19052	71217	3622	187954	6627	85210971	10659	94745	8.889	118493	70996	Biodegradation
258	61309	2526	83518	19233	72630	3681	189815	6725	87010432	10771	94976	8.818	118974	70979	Biodegradation
264	62499	2600	84924	19408	74040	3736	191666	6816	88767429	10879	95201	8.751	119440	70962	Biodegradation
270	63705	2675	86353	19594	75461	3793	193529	6898	90609320	10991	95419	8.681	119908	70930	Biodegradation
276	64891	2759	87752	19776	76853	3853	195350	6977	92448223	11102	95636	8.614	120373	70900	Biodegradation
282	66134	2827	89187	19954	78286	3922	197196	7056	94267564	11211	95857	8.550	120835	70878	Biodegradation
288	67374	2899	90624	20130	79719	3999	199029	7129	96084206	11319	96060	8.487	121278	70842	Biodegradation
294	68617	2978	92069	20300	81158	4073	200855	7199	97877026	11424	96245	8.425	121697	70793	Biodegradation
300	69835	3056	93488	20460	82564	4148	202637	7269	99594697	11524	96419	8.367	122094	70745	Biodegradation
306	71059	3142	94909	20597	83978	4227	204412	7337	101158346	11614	96584	8.316	122460	70709	Biodegradation
312	72277	3226	96329	20738	85382	4312	206156	7403	102777748	11706	96722	8.262	122804	70641	Biodegradation
318	73491	3314	97753	20873	86795	4390	207887	7464	104330526	11794	96830	8.210	123108	70552	Biodegradation
324	74668	3427	99139	21006	88185	4481	209569	7522	105929982	11884	96913	8.155	123392	70435	Biodegradation

FIGURE E-2 Difference Between the Means and 95% CI for the Linear Combination of 250 mg/kg Tolyltriazole and 1,900 mg/kg PG



APPENDIX F EXPERIMENT 2, MEAN CUMULATIVE OXYGEN CONSUMPTION CURVES AND 95% CONFIDENCE INTERVALS FOR EACH TREATMENT

The following five figures depict the 95% CI for the uncontaminated soil vs. each treatment in experiment two. The upper and lower CIs were determined using a t statistic based on a one sample t test using the equation seen below.

For a one sample t test, $t_{\alpha/2,n-1} = 4.303$
$CI = mean \pm 4.303 * Std\ Dev/(n)^{1/2}$

The null hypothesis was that there was not a significant difference between the uncontaminated and contaminated soils. In order for this hypothesis to be proven false, the CI for the uncontaminated and contaminated soils should not overlap.

List of Figures

FIGURE F-1 Mean Cumulative O_2 Consumption and 95% CI for the Uncontaminated and 25 mg/kg Tolyltriazole Contaminated High Clay Soil

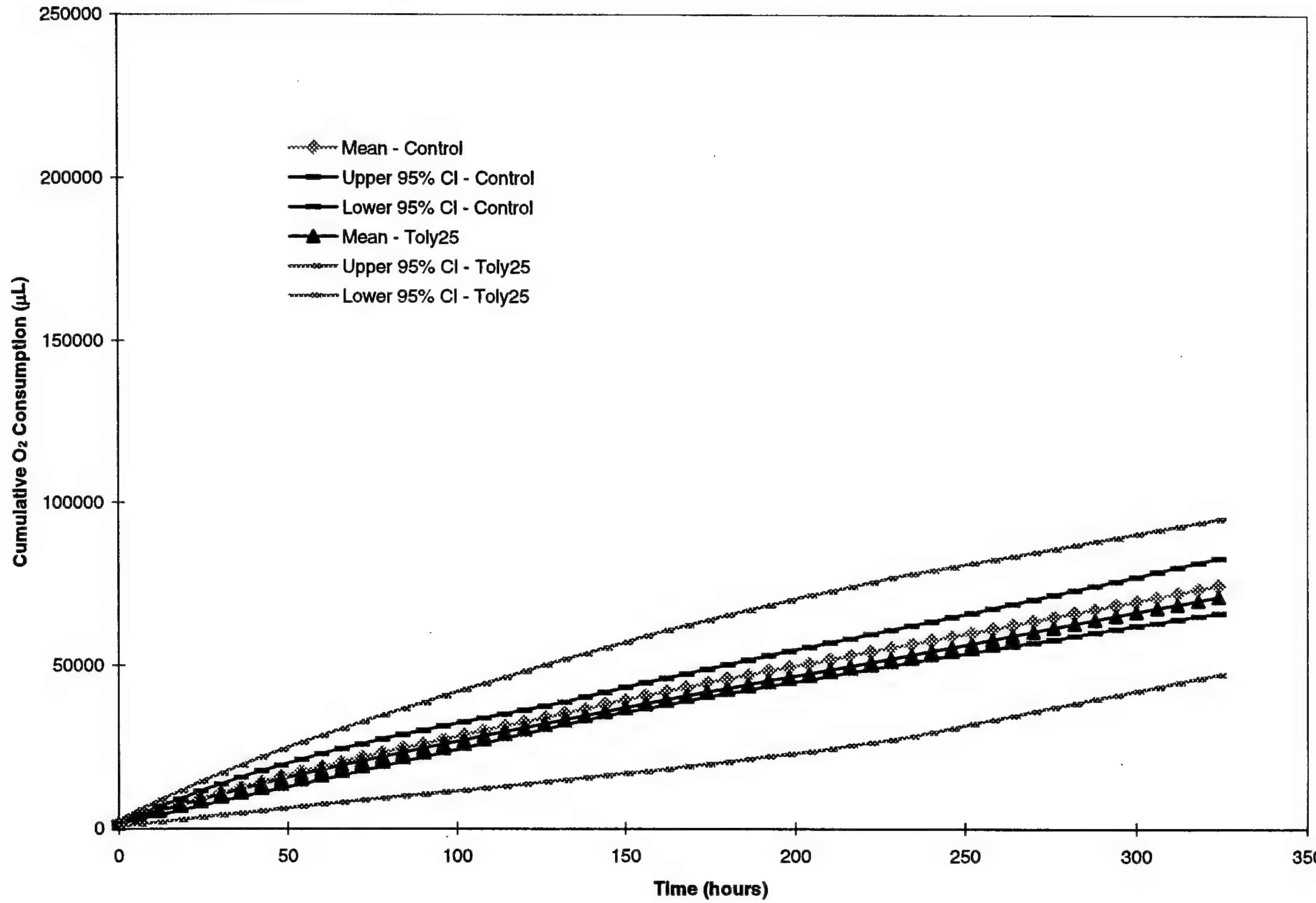


FIGURE F-2 Mean Cumulative O_2 Consumption and 95% CI for the Uncontaminated and 250 mg/kg Contaminated High Clay Soil

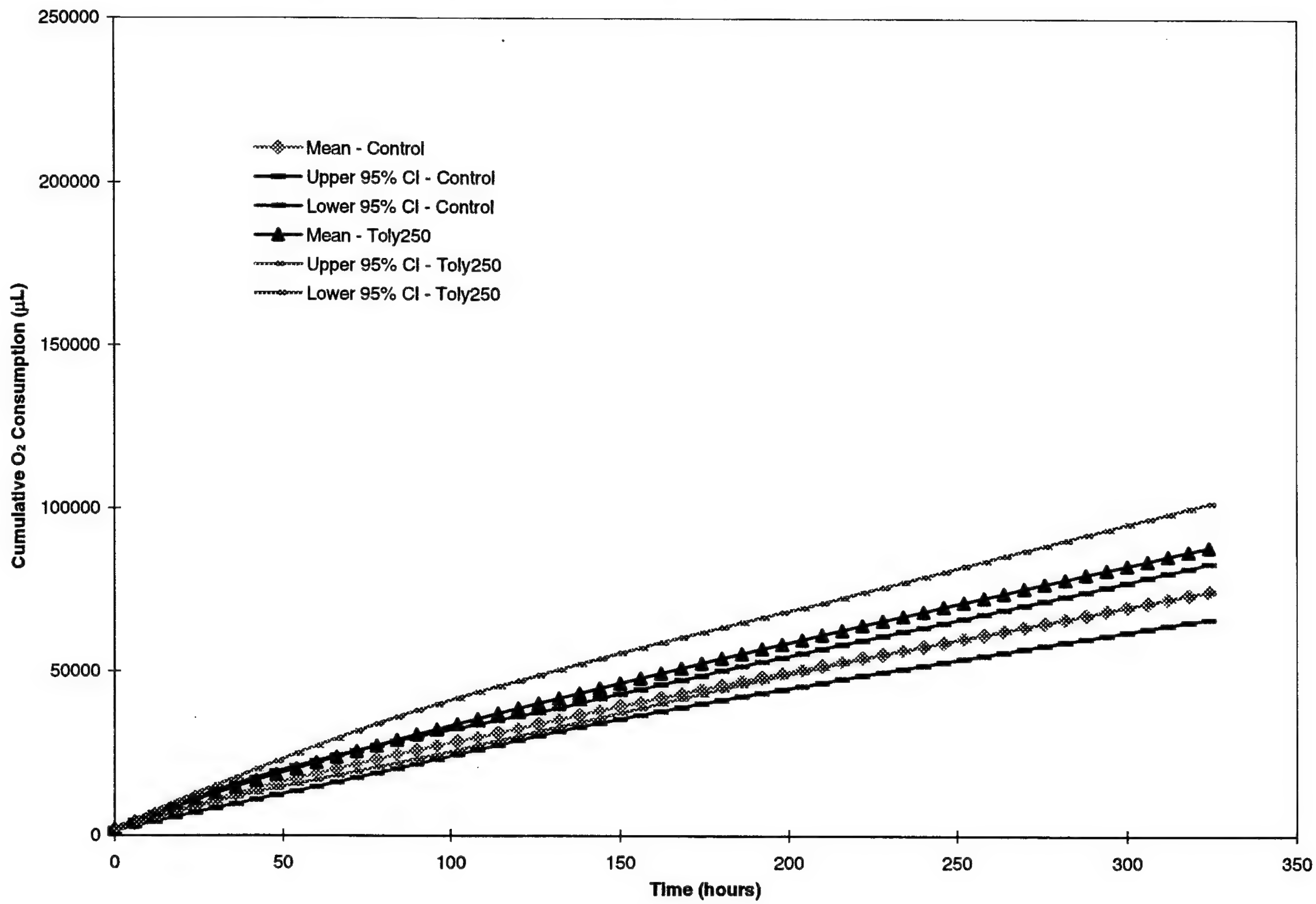


FIGURE F-3 Mean Cumulative O_2 Consumption and 95% CI for the Uncontaminated and 1,900 mg/kg PG Contaminated High Clay Soil

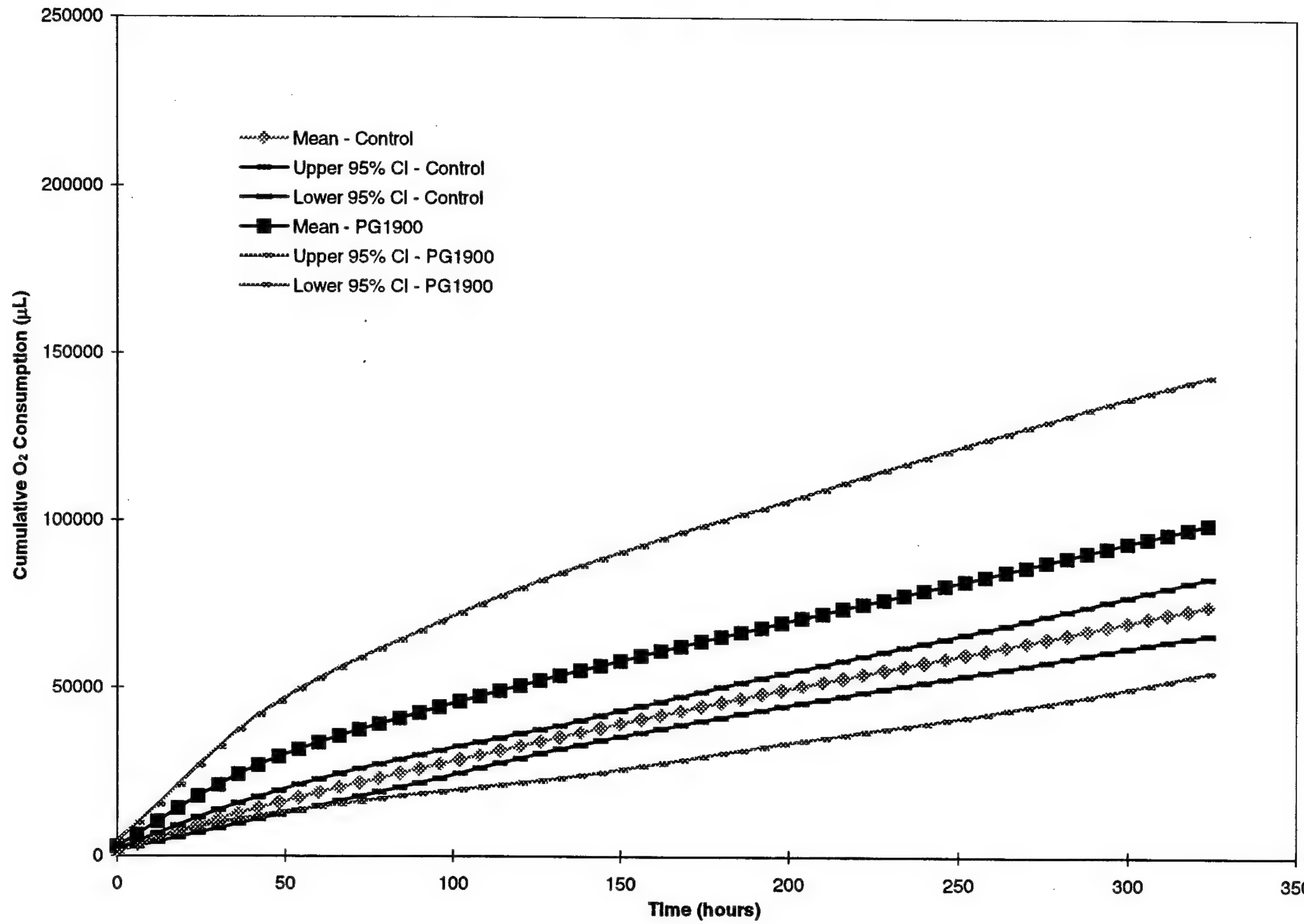


FIGURE F-4 Mean Cumulative O_2 Consumption and 95% CI for the Uncontaminated and Combined 25 mg/kg Tolyltriazole and 1,900 mg/kg PG Contaminated High Clay Soil

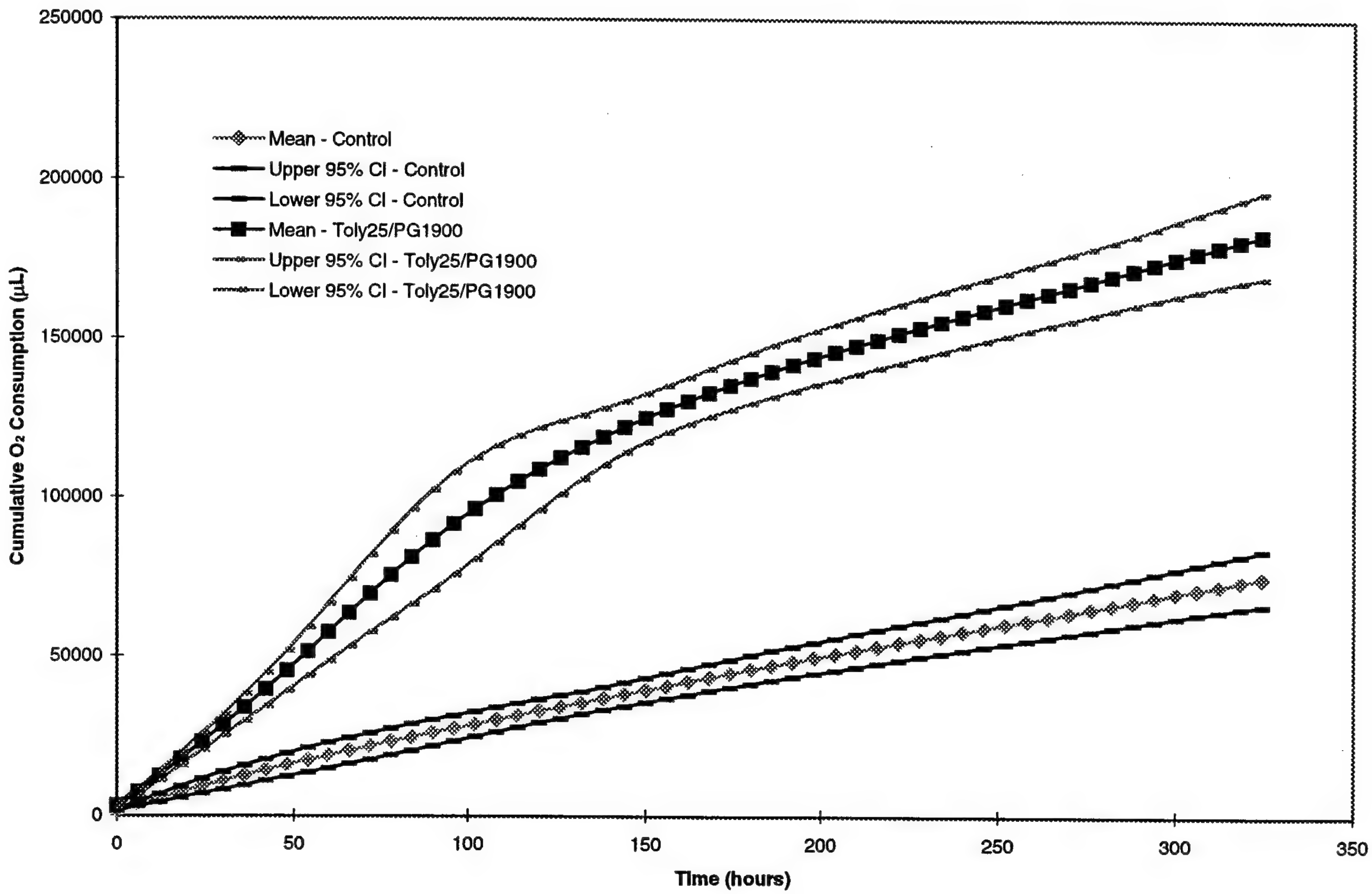
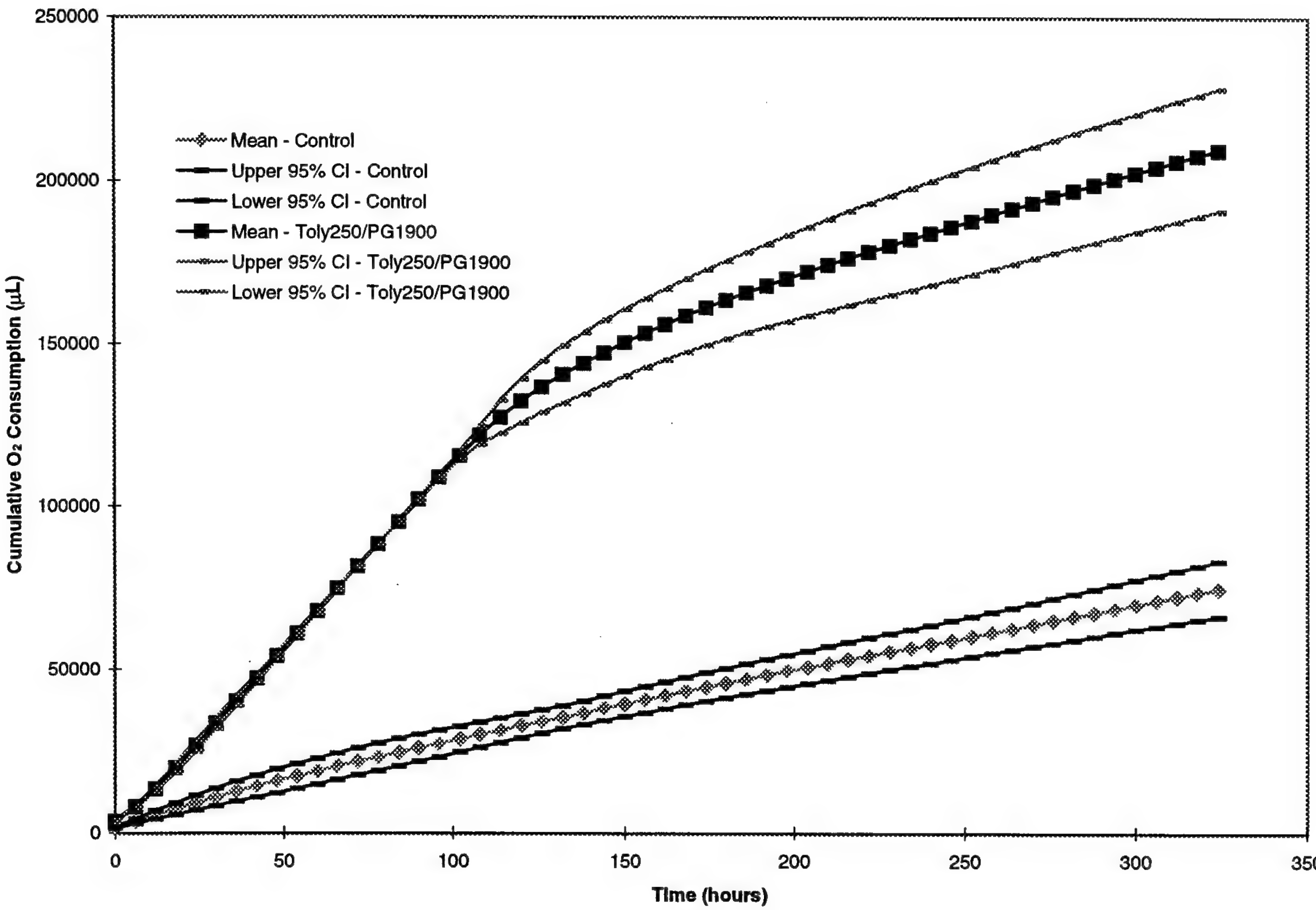


FIGURE F-5 Mean Cumulative O_2 Consumption and 95% CI for the Uncontaminated and Combined 250 mg/kg Tolyltriazole and 1,900 mg/kg PG Contaminated High Clay Soil



APPENDIX G HPLC RESULTS

The HPLC was used to detect the amount of tolyltriazole left in the microcosms upon completion of the respirometer tests. The tolyltriazole was extracted from the soil samples following the method described in chapter three. Because it was not known how well tolyltriazole could be extracted from the soil, a removal efficiency test was conducted as described in chapter four. The amount of tolyltriazole unrecovered is assumed to be lost to biodegradation.

Removal Efficiency

Added 100 g of wet soil to each microcosm.
Concentration of the contaminant = 2013 mg/L
Amount of contaminant added to the sandy soil = 5 mL
Amount of contaminant added to the high clay soil = 10 mL

High Clay Soil
Moisture content = 21.34%
Dry weight of the soil = 78.66 g
Amount of tolyltriazole added to the high clay soil microcosms = 255 mg/kg

Sandy Soil
Moisture content = 15.36%
Dry weight of the soil = 84.64 g
Amount of tolyltriazole added to the sandy soil microcosms = 120 mg/kg

TABLE G-1 Removal Efficiency: Weights Used in Calculations

Microcosm #	Wt of 40 mL Vial (g)	Wt Vial + Methanol (g)	Wt Vial + Soil + Methanol (g)	Wt of Methanol (g)	Wt of Soil (g)	Dry wt of Soil (g)	wt of H2O (g)
						wt soil-(mc*wt soil)	mc*wt soil
1 Sand	26.049	44.653	62.161	18.604	17.508	14.529	2.979
2 Sand	26.165	43.812	62.249	17.647	18.436	15.299	3.137
3 High Clay	26.108	44.440	59.343	18.331	14.904	10.057	4.847
4 High Clay	26.151	43.689	53.826	17.538	10.137	6.841	3.297

Moisture content of contaminated soil was computed as follows:

Total wt in microcosm = Dry wt of soil + moisture content + amt of H_2O and contaminant added

Total wt of liquid in microcosm = moisture content + amt of H_2O and contaminant added

New moisture content = total wt of liquid/total wt

Table G-2 below shows the calculations for the percent recovered, where the area of the peak came from the outputs of the HPLC.

TABLE G-2 Removal Efficiency: Calculations for Percent Tolyltriazole Recovered

Microcosm #	Area of Peak (mAu*s)	Conc. (mg/L)	Density of Meth/H_2O mix in Bottle	Mass of Toly in Bottle (mg)	End Conc (mg toly/kg soil)	% recovered of Original Conc
	y	y=9.487x	(wtH_2O+wtMeth)/(volH_2O+volMeth)	(conc/density)* (wt H_2O +Meth)		(end conc/init conc)*100
			density of meth=0.789		mg toly/kg soil	
1 Sand	539.216	56.834	0.812	1.509	103.884	87.364
2 Sand	555.560	58.556	0.814	1.493	97.607	82.085
3 High Clay	1416.825	149.335	0.825	4.193	416.959	162.921
4 High Clay	580.575	61.193	0.816	1.561	228.320	89.213
Stock Solution 100 mg/L	914.100	92.940				

Experiment 1

Added 100 g of wet soil to each microcosm.
Concentration of the contaminant = 2500 mg/L
Amount of contaminant added to each microcosm = 2 mL

High Clay Soil
Moisture content = 21.34%
Dry weight of the soil = 78.66 g
Amount of tolyltriazole added to the high clay soil microcosms = 65 mg/kg

Sandy Soil
Moisture content = 15.36%
Dry weight of the soil = 84.64 g
Amount of tolyltriazole added to the sandy soil microcosms = 60 mg/kg

TABLE G-3 Experiment 1: Weights Used in Calculations

Microcosm #	Wt of 40 mL Vial (g)	Wt Vial + Soil (g)	Wt Vial + Soil + Methanol (g)	Wt of Methanol (g)	Wt of Soil (g)	Dry wt of Soil (g)	wt of H2O (g)
						wt soil-(mc*wt soil)	mc*wt soil
2	26.266	40.270	57.304	17.033	14.004	9.449	4.554
3	26.197	40.085	54.799	14.713	13.888	9.371	4.516
4	26.085	42.145	59.282	17.137	16.059	10.836	5.222
9	26.289	40.194	56.661	16.467	13.905	9.383	4.522
12	26.075	49.932	57.511	7.579	23.856	19.797	4.059
15	26.244	46.215	56.761	10.546	19.970	16.572	3.398
18	26.073	50.787	63.180	12.392	24.714	20.509	4.205
19	26.375	50.909	60.393	9.484	24.533	20.359	4.174

Moisture content of contaminated soil was computed as follows:

High Clay

Total wt in microcosm = Dry wt of soil + moisture content + amt of H_2O and contaminant added

Total wt of liquid in microcosm = moisture content + amt of H_2O and contaminant added

New moisture content = total wt of liquid/total wt

Table G-4 below shows the calculations for the percent recovered, where the area of the peak came from the outputs of the HPLC.

TABLE G-4 Experiment 1: Calculations for Percent Tolyltriazole Recovered

Microcosm #	Area of Peak (mAu*s)	Conc. (mg/L)	Density of Meth/H_2O mix in Bottle	Mass of Toly in Bottle (mg)	End Conc (mg toly/kg soil)	% recovered of Original Conc
		y=9.835x	(wtH_2O+wtMeth)/(volH_2O+volMeth)	(conc/density)*(wt H_2O +Meth)		(end conc/init conc)*100
			density of meth = 0.789		mg toly/kg soil	
2 High Clay	105.401	10.717	0.826	0.280	29.647	46.637
3 High Clay	90.374	9.189	0.830	0.213	22.713	35.729
4 High Clay	77.263	7.856	0.830	0.212	19.530	30.723
9 High Clay	78.405	7.972	0.827	0.202	21.573	33.936
12 Sand	298.497	30.350	0.852	0.415	20.949	35.464
15 Sand	160.807	16.350	0.832	0.274	16.539	27.999
18 Sand	341.048	34.676	0.834	0.690	33.665	56.991
19 Sand	280.568	28.527	0.843	0.462	22.691	38.413

Experiment 2

Added 100 g of wet soil to each microcosm.
Concentration of the contaminant = 1980 mg/L
Amount of contaminant added to microcosms 1, 5, 11, 3, 8, 18 = 1 mL
Amount of contaminant added to microcosms 7, 9, 15, 12, 13, 16 = 10 mL

High Clay Soil
Moisture content = 21.34%
Dry weight of the soil = 78.66 g
Amount of tolyltriazole added to microcosms 1, 5, 11, 3, 8, 18 = 25 mg/kg
Amount of tolyltriazole added to microcosms 7, 9, 15, 12, 13, 16 = 250 mg/kg

TABLE G-5 Experiment 2: Weights Used in Calculations

Microcosm #	Wt of 40 mL Vial (g)	Wt Vial + Soil (g)	Wt Vial + Soil + Methanol (g)	Wt of Methanol (g)	Wt of Soil (g)	Dry wt of Soil (g)	Wt of H_2O (g)
						wt Soil-(mc*wt Soil)	mc*wt soil
1-Toly25	26.227	39.328	61.108	21.779	13.101	8.840	4.260
5-Toly25	26.271	39.253	64.274	25.021	12.981	8.759	4.221
11-Toly25	26.275	40.391	61.831	21.440	14.115	9.524	4.590
7-Toly250	26.370	38.590	59.498	20.907	12.219	8.245	3.974
9-Toly250	26.271	38.850	59.587	20.737	12.579	8.488	4.090
15-Toly250	26.290	40.305	55.519	15.213	14.014	9.457	4.557
3-PG/Toly25	26.206	42.977	64.823	21.846	16.770	11.316	5.454
8-PG/Toly25	26.253	41.808	63.292	21.483	15.555	10.496	5.058
18-PG/Toly25	26.275	40.785	63.092	22.306	14.510	9.791	4.718
12-PG/Toly250	26.267	42.296	63.820	21.524	16.028	10.816	5.212
13-PG/Toly250	26.180	37.27	57.187	19.910	11.096	7.487	3.608
16-PG/Toly250	26.257	40.687	61.670	20.983	14.429	9.736	4.692

Moisture content of contaminated soil was computed as follows:

Total wt in microcosm = Dry wt of soil + moisture content + amt of H_2O and contaminant added

Total wt of liquid in microcosm = moisture content + amt of H_2O and contaminant added

New moisture content = total wt of liquid/total wt

Table G-6 below shows the calculations for the percent recovered, where the area of the peak came from the outputs of the HPLC.

TABLE G-6 Experiment 2: Calculations for Percent Tolyltriazole Recovered

Microcosm #	Area of Peak (mAu*s)	Conc. (mg/L)	Density of Meth/H_2O mix in Bottle	Mass of Toly in Bottle (mg)	End Conc (mg toly/kg soil)	% recovered of Original Conc
	y	y=9.48754x	(wtH_2O+wtMeth)/(volH_2O+volMeth)	(conc/density)*(wt H_2O +Meth)		(end conc/init conc)*100
		9.487	density of meth=0.789		mg toly/kg soil	
1-Toly25	0	0	0.817	0	0	0
5-Toly25	56.286	5.932	0.813	0.213	24.336	96.748
11-Toly25	16.647	1.754	0.819	0.055	5.851	23.261
7-Toly250	521.680	54.985	0.816	1.675	203.190	80.778
9-Toly250	333.768	35.179	0.817	1.068	125.875	50.041
15-Toly250	593.713	62.578	0.829	1.491	157.740	62.709
3-PG/Toly25	18.13	1.910	0.823	0.063	5.596	22.247
8-PG/Toly25	0	0	0.822	0	0	0
18-PG/Toly25	0	0	0.819	0	0	0
12-PG/Toly250	444.012	46.799	0.822	1.520	140.583	55.888
13-PG/Toly250	345.074	36.371	0.815	1.049	140.101	55.697
16-PG/Toly250	402.392	42.412	0.820	1.326	136.276	54.176

APPENDIX H STATISTICAL DATA FOR O_2/CO_2 RATIO

The table and figure in this appendix show the average oxygen consumption, carbon dioxide evolution, and the ratio of the two numbers for each treatment in experiment 2. The figure shows the relationship of the ratios to one another for each treatment.

List of Tables

List of Figures

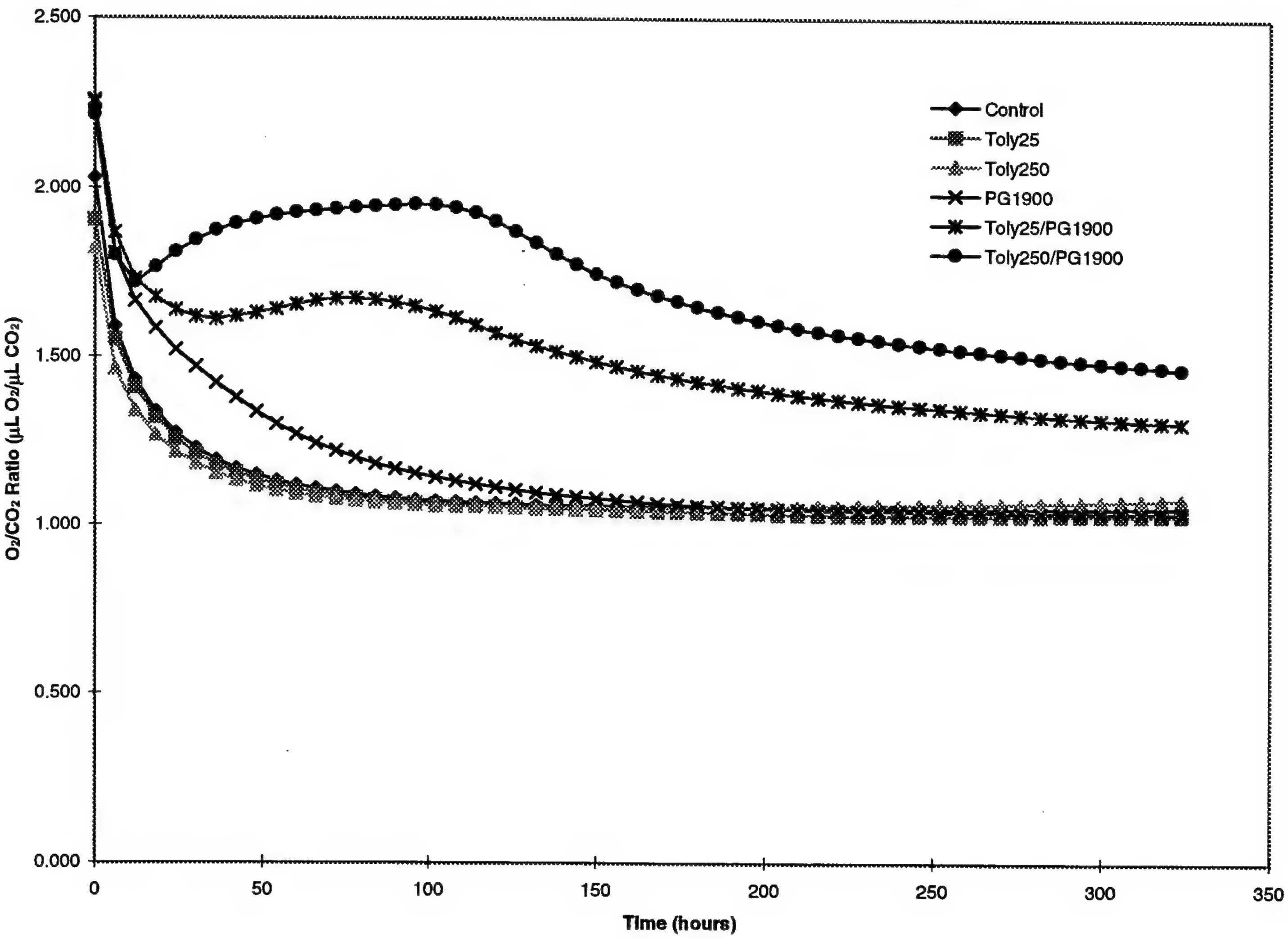
TABLE H-1 Experiment 2 O_2/CO_2 Ratio for Each Treatment

Time (hrs)	Control	Control	Control	Toly25	Toly25	Toly25	Toly250	Toly250	Toly250	PG1900	PG1900	PG1900	Toly25/PG 1900	Toly25/PG 1900	Toly25/PG 1900	Toly250/ PG1900	Toly250/ PG1900	Toly250/ PG1900
	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2
0	1717	845	2.031	1632	856	1.905	1924	1055	1.824	2930	1298	2.258	3289	1462	2.249	3604	1628	2.214
6	3624	2278	1.591	3550	2288	1.552	4148	2833	1.464	6444	3565	1.807	7579	4056	1.869	7996	4442	1.800
12	5515	3853	1.431	5404	3836	1.409	6374	4754	1.341	10235	6140	1.667	12491	7215	1.731	13501	7851	1.720
18	7384	5524	1.337	7189	5444	1.321	8587	6772	1.268	14092	8887	1.586	17796	10606	1.678	19860	11253	1.765
24	9207	7232	1.273	8888	7075	1.256	10726	8802	1.219	17724	11649	1.521	23045	14052	1.640	26591	14684	1.811
30	10972	8937	1.228	10550	8697	1.213	12799	10821	1.183	21155	14385	1.471	28372	17516	1.620	33464	18126	1.846
36	12631	10585	1.193	12108	10268	1.179	14755	12775	1.155	24235	17025	1.423	33793	20953	1.613	40354	21539	1.874
42	14256	12200	1.169	13626	11794	1.155	16680	14703	1.134	27003	19571	1.380	39458	24344	1.621	47232	24927	1.895
48	15840	13786	1.149	15117	13306	1.136	18552	16596	1.118	29439	22019	1.337	45318	27793	1.631	54131	28364	1.908
54	17376	15340	1.133	16565	14785	1.120	20376	18454	1.104	31671	24360	1.300	51339	31241	1.643	61020	31796	1.919
60	18850	16834	1.120	17955	16206	1.108	22133	20243	1.093	33717	26560	1.269	57387	34668	1.655	67884	35211	1.928
66	20330	18332	1.109	19344	17624	1.098	23928	22045	1.085	35686	28703	1.243	63560	38120	1.667	74728	38652	1.933
72	21758	19780	1.100	20686	18998	1.089	25670	23793	1.079	37535	30730	1.221	69554	41572	1.673	81590	42089	1.939
78	23174	21211	1.093	22013	20350	1.082	27392	25515	1.074	39323	32748	1.201	75415	45030	1.675	88439	45531	1.942
84	24548	22598	1.086	23295	21657	1.076	29062	27184	1.069	41025	34680	1.183	81021	48476	1.671	95292	48958	1.946
90	25921	23983	1.081	24587	22974	1.070	30721	28839	1.065	42721	36601	1.167	86419	51948	1.664	102131	52395	1.949
96	27270	25343	1.076	25862	24254	1.066	32349	30462	1.062	44376	38462	1.154	91503	55407	1.651	109052	55830	1.953
102	28642	26699	1.073	27140	25528	1.063	33977	32068	1.060	46029	40302	1.142	96304	58870	1.636	115673	59266	1.952
108	29982	28019	1.070	28383	26768	1.060	35566	33624	1.058	47608	42065	1.132	100746	62307	1.617	121803	62679	1.943
114	31349	29361	1.068	29651	28025	1.058	37174	35195	1.056	49202	43841	1.122	104911	65776	1.595	127469	66128	1.928
120	32688	30683	1.065	30890	29263	1.056	38747	36729	1.055	50734	45560	1.114	108746	69152	1.573	132423	69581	1.903
126	34033	32007	1.063	32131	30500	1.053	40322	38256	1.054	52258	47275	1.105	112357	72400	1.552	136742	73038	1.872
132	35332	33294	1.061	33326	31708	1.051	41854	39741	1.053	53708	48928	1.098	115714	75475	1.533	140575	76456	1.839
138	36702	34601	1.061	34559	32926	1.050	43421	41235	1.053	55209	50598	1.091	118959	78460	1.516	144116	79804	1.806
144	38052	35900	1.060	35782	34140	1.048	44972	42711	1.053	56696	52239	1.085	122018	81299	1.501	147354	83019	1.775
150	39397	37209	1.059	37019	35368	1.047	46534	44197	1.053	58207	53904	1.080	124949	84041	1.487	150421	86082	1.747
156	40713	38492	1.058	38232	36571	1.045	48080	45655	1.053	59673	55521	1.075	127700	86680	1.473	153302	88954	1.723
162	42031	39774	1.057	39452	37788	1.044	49633	47116	1.053	61148	57138	1.070	130337	89268	1.460	156067	91691	1.702
168	43326	41026	1.056	40658	38995	1.043	51157	48544	1.054	62596	58709	1.066	132828	91713	1.448	158690	94261	1.684
174	44617	42270	1.056	41853	40187	1.041	52672	49962	1.054	64028	60259	1.063	135231	94057	1.438	161201	96751	1.666
180	45859	43464	1.055	43009	41345	1.040	54135	51329	1.055	65405	61740	1.059	137504	96269	1.428	163551	99162	1.649
186	47092	44661	1.054	44159	42505	1.039	55599	52696	1.055	66779	63220	1.056	139697	98463	1.419	165832	101490	1.634
192	48315	45843	1.054	45309	43661	1.038	57050	54047	1.056	68151	64675	1.054	141821	100576	1.410	168062	103724	1.620
198	49523	47017	1.053	46445	44819	1.036	58487	55393	1.056	69547	66135	1.052	143884	102637	1.402	170239	105885	1.608

TABLE H-1 Experiment 2 O_2/CO_2 Ratio for Each Treatment

Time (hrs)	Control	Control	Control	Toly25	Toly25	Toly25	Toly250	Toly250	Toly250	PG1900	PG1900	PG1900	Toly25/PG 1900	Toly25/PG 1900	Toly25/PG 1900	Toly250/ PG1900	Toly250/ PG1900	Toly250/ PG1900
	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2	O_2	CO_2	O_2/CO_2
204	50662	48153	1.052	47525	45953	1.034	59854	56693	1.056	70929	67546	1.050	145833	104602	1.394	172317	107938	1.596
210	51879	49305	1.052	48672	47104	1.033	61306	58011	1.057	72353	68980	1.049	147816	106554	1.387	174403	109968	1.586
216	53077	50450	1.052	49808	48252	1.032	62737	59319	1.058	73760	70396	1.048	149760	108460	1.381	176434	111940	1.576
222	54268	51597	1.052	50938	49406	1.031	64167	60631	1.058	75170	71821	1.047	151675	110348	1.375	178433	113879	1.567
228	55433	52716	1.052	52056	50539	1.030	65572	61912	1.059	76547	73212	1.046	153533	112175	1.369	180376	115751	1.558
234	56612	53855	1.051	53218	51687	1.030	66998	63206	1.060	77940	74620	1.044	155388	113996	1.363	182308	117606	1.550
240	57777	54976	1.051	54390	52826	1.030	68404	64473	1.061	79324	76003	1.044	157211	115776	1.358	184202	119408	1.543
246	58951	56108	1.051	55582	53976	1.030	69820	65751	1.062	80729	77403	1.043	159044	117556	1.353	186097	121201	1.535
252	60122	57221	1.051	56773	55115	1.030	71217	66999	1.063	82114	78772	1.042	160845	119293	1.348	187954	122943	1.529
258	61309	58356	1.051	57968	56268	1.030	72630	68265	1.064	83518	80167	1.042	162644	121043	1.344	189815	124696	1.522
264	62499	59486	1.051	59170	57423	1.030	74040	69521	1.065	84924	81552	1.041	164430	122778	1.339	191666	126433	1.516
270	63705	60631	1.051	60381	58588	1.031	75461	70786	1.066	86353	82961	1.041	166222	124524	1.335	193529	128171	1.510
276	64891	61752	1.051	61571	59739	1.031	76853	72022	1.067	87752	84339	1.040	167977	126231	1.331	195350	129870	1.504
282	66134	62902	1.051	62792	60904	1.031	78286	73272	1.068	89187	85742	1.040	169759	127958	1.327	197196	131582	1.499
288	67374	64051	1.052	64019	62073	1.031	79719	74513	1.070	90624	87135	1.040	171595	129674	1.323	199029	133274	1.493
294	68617	65211	1.052	65246	63245	1.032	81158	75759	1.071	92069	88540	1.040	173470	131406	1.320	200855	134961	1.488
300	69835	66346	1.053	66451	64397	1.032	82564	76971	1.073	93488	89914	1.040	175321	133114	1.317	202637	136605	1.483
306	71059	67496	1.053	67657	65551	1.032	83978	78189	1.074	94909	91291	1.040	177180	134834	1.314	204412	138246	1.479
312	72277	68643	1.053	68860	66707	1.032	85382	79396	1.075	96329	92666	1.040	179023	136549	1.311	206156	139858	1.474
318	73491	69791	1.053	70057	67856	1.032	86795	80603	1.077	97753	94045	1.039	180862	138268	1.308	207887	141454	1.470
324	74668	70898	1.053	71234	68985	1.033	88185	81779	1.078	99139	95390	1.039	182668	139965	1.305	209569	143001	1.466

FIGURE H-1 Experiment 2 - Average O_2/CO_2 Ratio for Each Treatment



BIBLIOGRAPGHY

1. Baker, James A. Evaluation of the Natural Biodegradation of JP-8 in Various Soils using Respirometry. Air Force Institute of Technology Masters Thesis, 1995.

2. Cancilla, Devon A., Anke Holtkamp, Luca Matassa, and Xingchun Fang. "Isolation and Characterization of Microtox-Activated Components From Aircraft De-Icing/Anti-Icing Agents," Environmental Toxicology and Chemistry, 16(3): 430-434 (1997).

3. Cornell, Jeff, University of Colorado - Boulder, PhD Student, Boulder Colorado, Telephone interviews and e-mail, 1997.

4. Devore, Jay L., Probability and Statistics for Engineering and the Sciences. 4th ed, Duxbury Press, 1995.

5. Eaton, Andrew D., Lonore S. Clesceri, and Arnold E. Greenberg, Standards Methods for the Examination of Water and Wastewater. 19th ed, American Public Health Association, Washington D. C., 1995.

6. Fetter, C.W., Applied Hydrology, 3rd ed., Englewood Cliffs, NJ: Prentice Hall, 1994.

7. G. & C. Merriam Company, Webster's New Collegiate Dictionary. G. & C. Merriam Company, 1979.

8. Gallagher, Lynn M., Environmental Law Handbook, 13th ed., Ed, Thomas F.P. Sullivan, Rockville, MD: Government Institutes, Inc., 1995.

9. Gonsior, Stanley J. and Robert J. West. "Biodegradation of Glycol Ethers in Soil," Environmental Toxicology and Chemistry, 14(8): 1273-1279 (1995).

10. Haines, J.R., and M. Alexander, "Microbial Degradation of Polyethylene Glycols," Applied Microbiology. 29(5): 621-625 (1975).

11. Hartwell, Ian S., David Jordahl, Joyce Evans, and Eric May. "Toxicity of Aircraft De-Icer and Anti-Icer Solutions to Aquatic Organisms," Environmental Toxicology and Chemistry, 14(8): 1375-1386 (1995).

12. HQ Air Force Center for Environmental Excellence, PRO-ACT Fact Sheet, TI 6159, July 1995.

13. Human Systems Center Development Planning Directorate, Technology Assessment Requirements Analysis - Deicing Final Report, Contract No. F33615-90-D-0652/0006, October 23, 1996.

14. Jank, B.E., H.M. Guo, and V.W. Cairns. "Activated Sludge Treatment of Airport Wastwater Containing Aircraft De-Icing Fluids," Water Research, 8: 875-880 (1974).

15. Kaplan, David L., John T. Walsh, and Arthur M. Kaplan, "Gas Chromatographic Analysis of Glycols to Determine Biodegradability," Environmental Science and Technology. vol 16:723-725 (1982).

16. Kawai, Fusako, et al. "Bacterial Oxidation of Polyethylene Glycol," Applied and Environmental Microbiology. 35(4): 679-684 (1978).

17. Klecka, Gary M., "Biodegradation," in Environmental Exposure from Chemicals, vol 1, Ed, W. Brock Neely and Gary E. Blau, Boca Raton, FL: CRC Press Inc, 1995.

18. _____, Gary M., C.L. Carpenter, and B.D. Landenberger. "Biodegradation of Aircraft Deicing Fluids in Soil at Low Temperatures," Ecotoxicology and Environmental Safety, 25: 280-295 (1993).

19. Lokke, Hans. "Leaching of Ethylene Glycol and Ethanol in Subsoils," Water, Air, and Soil Pollution, 12: 373-387 (1984).

20. Mallinckrodt Laboratory Chemicals. Material Safety Data Sheet for 1,2-Propanediol (Propylene Glycol), Mallinckrodt Laboratory Chemicals. (1997).

21. Mericas, D. and B. Wagoner. "Balancing Safety and the Environment," Water Environment & Technology, 12: 38-43 (December 1994).

22. Metting, F. Blaine, Jr., "Structure and Physiological Ecology of Soil Microbial Communities," in Soil Microbial Ecology: Applications in Agricultural and Environmental Management. Ed, F. Blaine Metting, Jr., New York: Marcel Dekker Inc., 1993.

23. Micro-Oxymax v6.03 Instruction Manual. Publication Number 0135-5002. Columbus, OH: Columbus Instruments International Corportation, 1996.

24. Morrison, Robert T. and Robert N. Boyd. Organic Chemistry. 2nd ed., Boston: Allyn and Bacon, Inc., 1971.

25. NASA/FAC:237-11 "Air Force/NASA Biodegradable Deicing Fluid Formulation and Evaluation MOU Annex." Official Letter. Civil Technology Office. February 11, 1993.

26. Nitschke, L., H. Wagner, G. Metzner, A. Wilk, and L. Huber. "Biological Treatment of Waste Water Containing Glycols from De-Icing Agents," Water Resources. 33(3): 644-648 (1996).

27. O'Conner, Robert and Kate Douglas. "Cleaning Up After the Big Chill," New Scientist: 22-23 (16 January 1993).

28. Pillard, David A., "Comparative Toxicity of Formulated Glycol Deicers and Pure Ethylene and Proylene Glycol to *Ceriodaphnia Dubia and Pimephales Promelas*," Environmental Toxicology and Chemistry, 14(2): 331-315 (1995).

29. PMC Specialties Group. "COBRATEC (R) TT-100 Technical Bulletin," Technical Bulletin COR4317. PMC Specialties Group, Inc. (1996).

30. _____. Material Safety Data Sheet for COBRATEC (R) TT-100, PMC Specialties Group, Inc. (1996).

31. Sherwin Williams Chemicals. "Benzotiazole Tolyltriazole Technical Bulletin," Technical Bulletin 143. Sherwin Williams Chemical Company, 1986.

32. Sims, J.L., R.C. Sims, and J.E. Matthews. Bioremediation of Contaminated Surface Soils, EPA/600/9-89/073, Ada, Oklahoma: U.S. Environmental Protection Agency, 1989.

33. Strong-Gunderson, Janet M., Susan Wheelis, Susan Carroll, Michael Waltz, and Anthony Palumbo. "Degradation of High Concentrations of Glycols, Antifreeze, and Deicing Fluids," in Microbial Processes for Bioremediation, Ed, Robert E. Hinchee, Fred J. Brockman, and Catherine M. Vogel, Columbus-Richmond: Battelle Press, 1995.

34. Thomas, John D., Surfactant Enhanced Microbial Degradation of JP-8 Contaminated Soil. Air Force Institute of Technology Masters Thesis, 1996.

35. Totten, Christian A., The Use of Respirometry to Determine the Effect of Nutrient Enhnacement on JP-8 Biodegradability. Air Force Institute of Technology Masters Thesis, 1995.

Vita

Captain Laura M. Johnson was born on 28 November 1968 in Duluth, Minnesota. She graduated from Duluth East High School in 1987, and attended the University of Minnesota. She graduated in 1992 with a Bachelor of Science in Civil Engineering. She was commissioned as a 2nd Lieutenant in the US Air Force on 19 June 1992, and entered active duty on 17 October 1992. She attended the Basic Communications Officers Training Course at Keesler AFB, MS from 20 October 1992 until 17 March 1993. From 29 March 1993 until 1 July 1995, she worked at HQ Air Force Space Command, Peterson AFB, CO as a Command, Control, Communications, and Computer (C4) Plans and Architectures Staff Officer and as a C4 Infrastructure Resource Management Staff Officer. From 2 July 1995 until 21 April 1996, she worked as an Executive Officer at the Air Force Space Command Communications Support Squadron, Peterson AFB, CO. She was subsequently selected to study for her Masters of Science in Engineering and Environmental Management at the Air Force Institute of Technology (AFIT) from June 1996 until December 1997. Upon completion of the AFIT program, she will be assigned to the 8th Civil Engineering Squadron, Kunsan AB, ROK.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	December 1997	Masters Thesis

4. TITLE AND SUBTITLE
Evaluation of the Natural Biodegradation of Aircraft Deicing Fluid Components in Soils

5. FUNDING NUMBERS

6. AUTHOR(S)
LAURA M. JOHNSON, Capt, USAF

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Air Force Institute of Technology
2750 P Street
Wright Patterson AFB, OH 45433-7765

8. PERFORMING ORGANIZATION REPORT NUMBER
AFIT/GEE/ENV/97D-12

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
WL/MLSE
Wright Patterson AFB, OH 45433

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*
This research effort was conducted to analyze the biodegradation of propylene glycol (PG) and tolyltriazole in a sandy soil and a high clay soil. Both an automated respirometer and a high performance liquid chromatograph (HPLC) were used in the analysis. Two separate experiments were conducted. In the first experiment, one level of tolyltriazole was added to the soils to determine whether or not there was a difference in the biodegradation rates of tolyltriazole in the two soils. The respirometer results indicated that there was a significant difference between the respiration rates of the microorganisms in the two soil types, and the HPLC results indicated that biodegradation of the tolyltriazole was occurring in the microcosms. In the second experiment, only the high clay soil was used since it had a significantly higher respiration rate than the sandy soil. This experiment was conducted to determine the affect (inhibition, stimulation, or no effect) of a combined treatment of tolyltriazole and PG vs. the contaminants acting by themselves. The soil was treated with tolyltriazole alone, PG alone, and a combined mixture of the two. One level of PG was used throughout, and two levels of tolyltriazole were used, for a total of five different treatments. Both the respirometer and HPLC results indicated that biodegradation was occurring. The respirometer results indicated that there was a significant increase in the respiration rates of the microorganisms when the contaminants were mixed vs. by themselves, thereby indicating an increase in biodegradation. The HPLC results, however, indicated that the same amount of tolyltriazole was biodegrading whether it was in combination with PG or acting alone. These results may indicate that the significant increase in respiration was due to an increase in biodegradation of PG.

14. SUBJECT TERMS
Biodegradation, High Performance Liquid Chromatography, Oxygen Uptake, Propylene Glycol, Respirometry, Soil, Tolyltriazole

15. NUMBER OF PAGES
119

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL